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AMINO ACID AND GLUCOSE METABOLISM FOLLOWING ORAL AND INTRAVENOUS AMINO ACID ADMINISTRATION IN THE DOG

JEFFREY TEPLER

1982

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AMINO ACID AND GLUCOSE METABOLISM FOLLOWING ORAL AND INTRAVENOUS AMINO ACID ADMINISTRATION IN THE DOG

BY

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TABLE OF CONTENTS

Abstract i-iii

Introduction 1-27

I. Amino Acid Exchange in the Postabsorptive State 1-3

II. The Effect of Protein Ingestion on Amino Acid Metabolism 3-7

III. The Effect of Protein Ingestion on Glucose Metabolism 7-9

IV. Alanine 9-19

  A. Its Role as a Gluconeogenic Precursor 9-13

  B. The Glucose Alanine Cycle 13-19

V. The Effect of Insulin and Glucagon on Amino Acid Metabolism 19-22

VI. Intestinal Absorption of Amino Acids 22-23

VII. Influence of the Route of Amino Acid Administration on its Metabolism 23-25

VIII. Amino Acid Transport 25-27

Methods 27-33

Results 33-43

Discussion 43-51

Figure Legends 52-55

Figures and Tables 56-79

Bibliography 80-91

ABSTRACT

Amino acid and glucose metabolism was studied in 9 awake dogs in the postabsorptive state and after the ingestion of 0.7 gm/kg body wt of the amino acid mixture Freamine II$^{(R)}$. In the postabsorptive state, there was no net exchange of branched chain amino acids (BCAA) across the hepatic or extrahepatic splanchnic (EHS) tissues. Alanine was produced by the EHS tissues (1.8±0.8 μmoles/min•kg) and removed by the liver (4.8±0.7 μmoles/min•kg). For the other gluconeogenic amino acids, there was a net average flux of 6.2 μmoles/min•kg of amino acid nitrogen from the peripheral to the splanchnic tissues. Glucose was removed by the EHS tissues (0.8 ±0.1 mg/min•kg) and produced by the hepatic tissues (2.9±0.3 mg/min•kg).

Following ingestion, there was prompt intestinal absorption as reflected by the rapidly rising portal concentrations and EHS release of all amino acids present in the Freamine II$^{(R)}$ mixture. Over the 3 hr study, 100% of the ingested gluconeogenic amino acids and 80% of the BCAA were recovered in the portal blood. Cumulative EHS release of alanine exceeded the ingested dose by 47%, reflecting continued intestinal production of this amino acid in the fed state. The liver removed a total of 1736±166 μmoles/kg of alanine, a quantity threefold greater than the ingested dose and twofold greater than the net EHS alanine release, which comprises both the ingested load and the postcibal intestinal production of alanine. Net splanchnic alanine uptake was significant at all times and, in association with rising arterial levels, reflects stimulation of peripheral alanine production in the fed state. The hepatic uptake of other gluconeogenic amino acids was essentially equal to the ingested load of these amino acids. 44% of the absorbed BCAA was removed by the liver, while the remainder escaped the splanchnic bed to the periphery.

Over the 3 hr study, the net flux of gluconeogenic amino acids from the peripheral to the splanchnic tissues exceeded the splanchnic escape of BCAA by 890 μmoles/kg•3 hrs.

Hepatic and net splanchnic production, and intestinal removal of glucose were significantly stimulated after amino acid ingestion. Despite this, plasma arterial glucose levels dropped 7 mg/dl below basal by the study's end. In 4 dogs, total body glucose turnover was measured isotopically using ^{3}H-3-glucose. Although the rate of glucose appearance (Ra), disappearance (Rd), and metabolic clearance (MCR) all rose after feeding, the rise in Rd exceeded that in Ra, resulting in a fall in arterial glucose levels. Mean portal glucagon levels rose 2½ fold and remained persistently elevated for the duration of the study. Mean portal and arterial insulin rose significantly but returned to baseline levels by 90 min.

Amino acid and glucose metabolism was also studied in dogs receiving a 3 hr primed-continuous infusion of Freamine II$^{(R)}$. Five dogs received a low-dose infusion (0.7 gm/kg), a dose equivalent to that administered in the oral studies, and 5 dogs received a high-dose infusion (1.4 gm/kg). Over the 3 hr low-dose and high-dose infusions, the liver removed quantities of alanine and other gluconeogenic amino acids which equalled or exceeded their infused loads, but there was no significant change in the handling of these amino acids by the EHS tissues. During the high-dose infusions, the hepatic and EHS tissues removed comparable quantities of BCAA and together metabolized 973±253 μmoles/kg or 37% of the administered load of these amino acids. Over the 3 hr high-dose infusion, the splanchnic tissues metabolized 51 μmoles/kg•min of administered amino acids, while the peripheral tissues removed 7 μmoles/kg•min, the latter quantity

clearly inadequate to replenish muscle nitrogen stores. The hepatic extraction ratios for all amino acids rose during the low and high-dose infusions, as following oral ingestion, and their values were strikingly similar in all 3 groups of dogs. The effect of parenterally administered amino acids on glucose metabolism and insulin and glucagon levels was very similar to that observed following oral ingestion.

It is concluded that, following ingestion of an amino acid mixture, (1) there is quantitative recovery in the portal blood of nearly all the ingested amino acids; (2) peripheral production of alanine is stimulated, while intestinal production continues at a rate similar to that observed in the postabsorptive state; (3) the liver removes a significant percentage of the ingested BCAA, the remainder escaping from the splanchnic bed to the periphery; (4) net postabsorptive flux of amino acid nitrogen from the peripheral to the splanchnic tissues persists in the fed state; (5) insulin and glucagon levels and the rates of hepatic production and peripheral uptake of glucose all rise, and there is a small fall in arterial glucose levels.

Following intravenous amino acid administration, (1) the splanchnic bed plays a pivotal role in amino acid disposal; (2) the liver removes gluconeogenic and branched chain amino acids, while the EHS tissues metabolize only the BCAA; (3) only a small percentage of the infused load is metabolized by peripheral tissues, a quantity inadequate to replenish muscle nitrogen stores; (4) hepatic extraction of amino acids is independent of the route of their administration.

INTRODUCTION

I. Amino Acid Exchange in the Postabsorptive State

It has long been suspected that muscle, the body's primary repository of protein, and the liver, its repository of urea cycle enzymes and major site of amino acid degradation, play a critical role in the maintenance of the relatively constant amino acid concentrations that persist in the postabsorptive state (i.e., following a 12-14 hour fast) in man.

Muscle. Release of amino acids by skeletal muscle in man in the postabsorptive state was first documented by London et al (1), who noted consistently negative arteriovenous differences for 11 of 16 amino acids across the deep venous bed of the human forearm. Output of alanine and glutamine exceeded that of all other amino acids, and alanine accounted for 30% of the total alpha amino nitrogen release (2). Pozefsky, et al (3), confirmed the primacy of alanine in peripheral amino acid release and found a correlation between net skeletal muscle output and ambient arterial concentrations of measured amino acids. Since alanine comprises no more than 10% of skeletal muscle tissue (2), it was postulated that alanine release reflects not only protein degradation, but de-novo synthesis of alanine by transamination of pyruvate. While most amino acids, including the branched chain amino acids (BCAA) - leucine, valine, and isoleucine - exhibit a net release from muscle in the postabsorptive state, glutamate and cystine were consistently taken up (4).

Splanchnic Tissues. Since the portal vein is inaccessible to catheterization in normal man, there have been few studies directly assessing the role of the liver in the metabolism of circulating

amino acids. Assessment of the contribution of the splanchnic bed as a whole, i.e., the hepatic and extrahepatic splanchnic tissues (spleen, pancreas, and upper intestinal tract), has principally involved the measurement of arterial-hepatic venous (A-HV) differences (5, 6). These studies have shown that, for the most part, splanchnic uptake of amino acids closely complements their skeletal muscle release. Alanine and glutamine contribute most prominently to splanchnic amino acid uptake, and alanine alone accounts for half of the total extraction. The BCAA, however, shows no consistent splanchnic uptake.

Studies of arterial-portal venous differences in human subjects catheterized while undergoing elective cholecystectomy (7, 8) and in experimental animals (2, 9) have shed light on the individual roles of the various splanchnic tissues in amino acid metabolism in the postabsorptive state. These studies have revealed that the gut releases substantial quantities of glucogenic amino acids and that its output of alanine is nearly equal to the alanine load presented to the liver from the periphery. Thus, measurement by A-HV differences of the overall splanchnic uptake of alanine had greatly underestimated the true uptake of this amino acid by the liver. In addition, these studies showed that the gut, rather than the liver, is solely responsible for glutamine uptake by splanchnic tissues. Studies in rats (10, 11, 12) have since identified glutamine and ketone bodies as the major respiratory fuels of the postabsorptive intestine. Furthermore, greater than 75% of the nitrogen taken up as glutamine by the small intestine can be accounted for by release as alanine and ammonia (12). There is no consistent uptake by either the liver or

the intestine of BCAA (6, 7). These findings are in accordance with studies in animals (2) which suggest that extrahepatic tissues are the major sites of BCAA catabolism. Finally, in the postabsorptive state in man, the net alpha amino nitrogen balance across muscle is negative, there being a net flux of amino acids to the splanchnic region (4, 7, 19).

Other Tissues. The other major organs in man which contribute significantly to amino acid metabolism are the kidney and liver. In the postabsorptive state in man, the kidney extracts glutamine as a source of nitrogen for ammoniagenesis, a process greatly accelerated during acidosis (13). It also releases alanine at a rate which is 10-20% of that found in muscle. The brain in the postabsorptive state extracts BCAA, particularly leucine, at a rate which exceeds that of all other amino acids (2). In fact, studies with rat tissue slices (14) have revealed that brain tissue catabolizes leucine several times more rapidly than does skeletal muscle tissue. However, since skeletal muscle constitutes 43% of the body's mass whereas the brain represents only 1%, muscle is probably the major site of BCAA catabolism in mammals (14).

II. The Effect of Protein Ingestion on Amino Acid Metabolism

That a protein meal is followed by an increase in the concentration of total circulating free amino acids was first shown by Van Slyke and Meyer (15) in 1912 in their experiments on dogs. With the advent of ion-exchange chromatography, Frame (16) quantitated free amino acid levels in humans after ingestion of 60 g of a high protein meal and found that, although most of the amino acids increased in concentration after the meal, the rises did not parallel the rel-

ative amino acid composition of the food. Most marked was the rise in the BCAA - valine, leucine, and isoleucine - levels of which remained elevated for 8 hr. after ingestion. Several studies in animals (9, 17, 18, 19) and man (20) have since underscored the unique tendency of these amino acids to escape splanchnic metabolism and to increase in arterial concentration after amino acid ingestion. Wahren et al (20) studied splanchnic and leg exchange of amino acids in seven healthy human subjects after the ingestion of a protein-rich meal. They found that, although there was a release of all amino acids from the splanchnic bed after the meal, the BCAA, which constituted only 20% of the amino acid residues in the meal (21), together accounted for more than half of the total splanchnic output over the three hours post-ingestion. Leg exchange for most amino acids reverted from a net release in the postabsorptive state to a net uptake after ingestion. Complementing the prominence of the BCAA in their escape from the splanchnic bed and rise in arterial concentration (100-200% above basal) was their striking uptake by the leg, accounting for greater than half of the total peripheral amino acid extraction after the meal. This finding confirmed that of Aoki et al (22), who studied amino acid metabolism across the forearm of a nitrogen-depleted subject after a protein meal and similarly found the BCAA to predominate among amino acids extracted by muscle.

Wahren et al (20) found, furthermore, that alanine and glutamine were unique among amino acids in their consistent net splanchnic uptake as well as in their persistent peripheral release after protein ingestion. The aforementioned studies suggested a unique

role for the BCAA in muscle nitrogen repletion after protein ingestion. Furthermore, inasmuch as BCAA constitute only 20% of muscle amino acids (22) but the majority of protein-induced peripheral amino acid uptake, it has been proposed that, in addition to being incorporated into muscle protein, they are locally catabolized and serve as the major source of nitrogen for the glucose-alanine cycle (7, 22, 23, 24), discussed below. Finally, Wahren et al (20) found that, despite the splanchnic release of BCAA to the periphery, there was a net flux of amino acid nitrogen from muscle to the splanchnic tissues during the 3 hours following protein ingestion. Aoki et al (76) studied amino acid exchange across the splanchnic bed and forearm of four healthy human subjects after the ingestion of ground sirloin. Their findings were strikingly similar to those of Wahren et al (20).

Due to the inaccessibility of the portal vein to catheterization in man, there is no data on the individual roles of the liver and intestine in the handling of an amino acid meal. However, studies in chronically catherized dogs (9), rats (17), and sheep (18) fed protein meals have furthered our understanding of their functions in the fed state. Several aspects of the disposal of a protein meal were consistently observed in all three species. Intestinal output of alanine exceeded that of all other amino acids. Gut release of BCAA greatly exceeded its extraction by the liver, resulting in a net splanchnic release to the periphery. The liver largely removed most other amino acids added to the portal blood, and thus prevented significant net splanchnic release of these substances. Finally, aspartate and glutamate, although constituting

20-30% of the ingested meal (2), appeared in only negligible quantities in the portal circulation. Also, a large portion of ingested glutamine was metabolized by the portal-drained viscera. Studies in fed rats (11) have since confirmed the latter findings, and it has been estimated that all dietary aspartate and glutamate and two-thirds of the glutamine are metabolized to urea precursors, primarily alanine and ammonia, during intestinal transport. It has been postulated that glutamine is preferentially metabolized in the gut rather than in the liver, the repository of urea cycle enzymes, due to the higher Km of hepatic glutaminase as compared with intestinal glutaminase. In addition, intestinal catabolism of ingested aspartate and glutamate, and glutamate derived from glutamine, prevents access of these neurotoxic (11) amino acids to the peripheral circulation.

The physiologic significance of the aforementioned studies (9, 17, 18) is uncertain in view of flaws in their experimental design. In the rat studies of Yamamoto et al (17), hepatic blood flow was not measured so that analysis of amino acid metabolism is qualitative rather than quantitative. In addition, the rats were studied while anaesthetized, the effects on amino acid metabolism of which are unknown. Finally, substrate measurements were performed on plasma, and studies by Felig et al (8) and Wahren et al (25) have shown that blood cellular elements contribute substantially to the interorgan transfer of amino acids (discussed below). In the sheep studies of Wolff et al (18), too, plasma was used for analysis. In addition, sheep, being ruminants, absorb little dietary glucose and depend heavily on hepatic gluconeogenesis for glucose production. This pro-

cess is considered to be greatly stimulated after ingestion of glucogenic amino acids and, thus, the relevance of these studies to nonruminant species is uncertain. The studies of Elwyn et al (9) were performed on but three animals, and ,within this small group, there were significant quantitative and at timesdirectional differences in the interorgan movements of the amino acids.

In their recent studies on dogs, Bloomgarden et al (19) have reported a significant correlation between gut output and hepatic uptake of amino acids after protein ingestion. They, too, noted a predominance of alanine amongst amino acids released from the gut. Its quantity exceeded that present in the ingested meal, suggesting continued intestinal alanine synthesis in the fed state. Hepatic extraction of alanine rose concomitant with the increase in gut output, accounting for its minimal net splanchnic output. Although peripheral balances were not measured, the rise in arterial alanine concentration despite continued splanchnic uptake suggests persistent peripheral production of alanine in the fed state, confirming the findings of Wahren et al (20) in humans. Finally, they noted a failure of the liver to take up ingested BCAA, and supported a purely passive role for the liver in the handling of these amino acids.

One of the major objectives of the current study was to quantitatively and definitively elucidate the individual roles of the hepatic and extrahepatic splanchnic tissues in the metabolism of an amino acid meal.

III. The Effect of Protein Ingestion on Glucose Metabolism

It has been shown that the opposing actions of insulin and glucagon are responsible for the fine regulation of hepatic gluco-

neogenesis and glucose homeostasis in the basal state (26). In addition, it has long been known that there is a rise in both insulin and glucagon secretion after protein ingestion (27, 28). The rise in insulin concentration has been proposed as a mechanism for facilitating peripheral uptake and utilization of ingested amino acids which escape splanchnic metabolism. Unger et al (29) have postulated that the purpose of aminogenic hyperglucagonemia is to stimulate hepatic glucose production, thereby preventing a decline in blood glucose concentration that would otherwise occur from unopposed protein-stimulated insulin secretion during carbohydrate-free protein meals. They have demonstrated that when endogenous glucagon secretion is suppressed by continuous glucose infusion, hyperaminoacidemia induces a mean maximal fall in glucose concentration of 35 mg/dl, significantly greater than the 3 mg/dl fall observed when endogenous glucagon production is not suppressed. The physiologic importance of aminogenic hyperglucagonemia is uncertain, however.

Wahren et al (20) have studied the effect of protein ingestion on splanchnic glucose metabolism in man and have found that in healthy subjects both the splanchnic production and the arterial concentration of glucose remain essentially unchanged after protein intake. In addition, they found that protein ingestion results in a rise in both insulin and glucagon arterial levels. Earlier studies by Felig and Wahren (5) have shown that when increments in arterial insulin comparable to those observed after protein ingestion (i.e., 60-100% above basal) are induced in normal postabsorptive man by glucose infusion, splanchnic glucose production falls

by 85%. Their data further suggested that the liver rather than the peripheral tissues is the primary target organ whereby glucose homeostasis is achieved with small increments in insulin. The fact that decline in splanchnic glucose production did not occur after protein ingestion is probably the result of concomitant aminogenic hyperglucagonemia. In this context, it is of interest to note that when Wahren et al (20) administered the protein meal to diabetics, there was a 150% rise in splanchnic glucose production concomitant with a two-fold rise in arterial glucagon levels. The authors speculate that the absence of a rise in insulin levels after the meal leads to unopposed hyperglucagonemia and increased hepatic glucose production in these patients.

The effect of a protein meal on glucose exchange across the liver and intestine has never been examined. In the current studies, this effect was assessed by the organ balance technique (see Methods). In addition, the rates of endogenous production, disappearance and metabolic clearance of glucose after protein ingestion were measured isotopically with 3-^{3}H-glucose (see Methods).

IV. Alanine

A. Its Role As A Gluconeogenic Precursor

The glucose produced by the liver derives from either glycogenolysis or gluconeogenesis. In the postabsorptive state, 75% of hepatic glucose production is the result of glycogen breakdown, while 25% is the product of gluconeogenesis (23). Lactate, pyruvate, glycerol, and amino acids, predominantly alanine, constitute the major substrates for the latter process. Alanine alone accounts for 25-40% of the gluconeogenic substrate (23). In situations where

glycogen stores are depleted, such as after a 24 hour fast (23) or prolonged exercise (2, 23), gluconeogenesis assumes primacy as the mode of glucose production, and alanine becomes all the more important as its primary substrate.

The importance of alanine as a gluconeogenic precursor was first suggested by Ross et al (30) who measured the rate of gluconeogenesis from all major physiologic precursors in the perfused rat liver and found that of the amino acids studied, the rates of glucose production were greatest from alanine, serine and proline. Mallette et al (31) subsequently reported that glucose production from alanine increased linearly with an increase in its concentration up to 9 mM or 20 times its normal physiologic concentration. Although later in vivo rat studies by Aikawa et al (32) noted linearity up to only 3-4 mM, the catalytic potential of this gluconeogenic pathway appears to be quite striking. Felig et al (33) have shown that the administration of exogenous alanine to starved, glycogen-depleted human subjects resulted in a prompt hyperglycemic response. They further showed (34) that human subjects, both in the postabsorptive state and after a prolonged fast, promptly incorporated ^{14}C from administered alanine-^{14}C into blood glucose with recovery in 30-60 minutes. On the basis of the above observations and the documentation of its prominent peripheral release and hepatic extraction (discussed above), alanine has been assigned a primary role as a gluconeogenic precursor.

The roles of insulin and glucagon in the regulation of gluconeogenesis from alanine have been extensively studied in recent years by either arterio-hepatic venous (A-HV) catheterization or tracer studies (5, 26, 31, 35-43), each technique possessing cer-

tain limitations (35). The A-HV catheterization technique provides accurate information on the splanchnic extraction rate of various gluconeogenic precursors but no information on the fate of the extracted precursor, i.e., whether it is converted to glucose. With the tracer technique, precursor fate can be determined, but, without complicated kinetic analysis, quantitative information regarding the conversion of precursors to glucose cannot be derived.

Felig et al (5), employing the A-HV catheterization technique, have reported that after glucose infusion in man, there is a rise in the arterial insulin level associated with a fall in the splanchnic extraction of alanine. They have further suggested that the insulin rise caused a decrease in hepatic alanine extraction with a resultant reduction in splanchnic glucose production. Subsequent studies by Chiasson et al in dogs (36) and man (37) have demonstrated that, when euglycemia is maintained by variable glucose infusion, a rise in insulin concentration does not alter splanchnic extraction of alanine. The authors have attributed the divergent findings of Felig et al (5) to the concomitant hyperglycemia in the latter study. In this regard, Shulman et al (33) have recently shown in dogs that glucose per se, i.e., independent of changes in insulin and glucagon secretion, can significantly impair hepatic fractional extraction of alanine. In the recent studies of Chiasson (37), a combination of the A-HV catheterization and the tracer techniques was employed in the assessment of the effects of insulin infusion at two doses on gluconeogenesis from alanine. Glucagon was infused for forty minutes at the study's end to flush out and quantitate any ^{14}C-glucose that may have accumulated in glycogen during the ^{14}C-alanine infu-

sion. They found that, after a 48 hour fast, low dose insulin infusion (with a resultant mean arterial insulin of 100 μU/ml) suppressed hepatic glucose production primarily by diverting newly synthesized glucose into glycogen while the high dose infusion (421 μU/ml, mean insulin level) inhibited glucose production by inhibiting gluconeogenesis from alanine.

That glucagon stimulates gluconeogenesis from alanine was first demonstrated by Garcia et al (39) and Mallette et al (31) in their studies on perfused rat livers. Livers perfused with glucagon showed a high glucose output which was largely a result of stimulated glycogenolysis. Chiasson et al (40), using tracer techniques, studied the effect of glucagon on gluconeogenesis in anesthetized dogs and found that, after the infusion of glucagon at a rate sufficient to produce arterial glucagon levels in the high physiologic range, gluconeogenesis from alanine was significantly stimulated. Tracer studies by Brockman et al (41) in sheep confirmed the stimulatory effect of glucagon on gluconeogenesis from alanine and documented a marked increase in hepatic extraction of alanine with a concomitant decline in its arterial concentration. Jennings et al (26) studied the roles of glucagon and insulin in the regulation of gluconeogenesis from alanine in the postabsorptive anesthetized dog using the tracer technique. By infusing somatostatin, a potent inhibitor of both pancreatic hormones, alone or in combination with the intraportal replacement infusion of either insulin or glucagon, a deficiency of either or both hormones was affected. They found that isolated insulin deficiency (basal glucagon levels maintained) caused a 74% increase and iso-

lated glucagon deficiency (basal insulin levels maintained) a 45% decrease in the conversion of alanine to glucose. Isolated deficiency of either hormone did not alter splanchnic extraction of alanine. This study demonstrated the existence of a delicate balance between the inhibitory effect of insulin and the stimulatory effect of glucagon in the maintenance of basal gluconeogenesis. It furthermore suggests, in contradistinction to earlier studies (5, 23, 31, 40), that both hormones influence gluconeogenesis from alanine not by altering its hepatic extraction but by regulating its intrahepatic metabolism.

The observation that glucagon production is markedly stimulated in man during states of increased gluconeogenesis (23, 42) has suggested that the hormone is an important regulator of the latter process in humans. Following a 72 hour fast, for example, alanine administration results in an increase in plasma glucose that is proportional to the rise in plasma glucagon (42). Chiasson et al (43), combining the A-HV catheterization and the tracer techniques, have reported that, in postabsorptive man, after the administration of supraphysiologic doses of glucagon, gluconeogenesis from alanine is doubled. In addition, hepatic alanine extraction is unaltered, confirming their earlier findings in dogs (26) that the hormone exerts a purely intrahepatic stimulatory effect on gluconeogenesis. The importance of glucagon at physiologic levels in the regulation of gluconeogenesis in man remains unresolved.

B. The Glucose-Alanine Cycle

Alanine constitutes 7-10% of muscle amino acid residues (2, 21) but greater than 30% of the total peripheral amino acid release

(2, 3). This led Felig et al (3, 7, 23) and Mallette et al (31) to independently postulate that alanine is synthesized de novo in muscle by the transamination of pyruvate, utilizing amino groups generated by local amino acid degradation. The BCAA, which constitute 20% of muscle protein but only 10-12% of the amino acid nitrogen released, were postulated to be the source of the amino groups. It was hypothesized that alanine released by skeletal muscle helped to transport the toxic ammonia products of BCAA catabolism to the liver for disposal as urea while serving as a major gluconeogenic precursor for the liver. Many clinical and biochemical observations have since underscored the physiologic importance of the glucose-alanine cycle and elucidated the origin of newly synthesized alanine in skeletal muscle.

The origin of the carbon skeleton of alanine released by skeletal muscle has been a topic of lively contention for many years. Many investigators (21, 45, 49-52), in support of the glucose-alanine cycle, have postulated that the carbon skeleton is derived almost exclusively from glycolysis of exogenous glucose and not from amino acids generated by local protein degradation. Others (46-48) have suggested that the carbon skeleton is derived primarily from local catabolism within the muscle cell with subsequent conversion of the carbon skeleton to pyruvate via the tricarboxylic acid (TCA) cycle.

Odessey et al (21) first demonstrated that when ^{14}C-glucose is added to the diaphragms of fasted rats in vitro, at least 60% of the alanine contains carbon from the labelled precursor. The addition of BCAA increased alanine production two-fold with at least 70% of

alanine's carbon coming from labelled exogenous glucose. In addition, alanine production from fasted rat diaphragms increased upon addition of glucose and even further in the presence of insulin. The further stimulation with insulin presumably reflected its ability to facilitate transport and/or metabolism of glucose since by itself insulin had no effect on alanine synthesis. The experiments of Manchester (49) and Odessey et al (45) on rat diaphragms and later studies by Chang and Goldberg (50) on rat soleus and extensor digitorum showed that, of all amino acids examined, only ^{14}C-labelled aspartate, glutamate, valine, isoleucine and asparagine were converted to $^{14}CO_2$. The lack of $^{14}CO_2$ production from other labelled amino acids indicates that they cannot be converted to intermediates in the TCA cycle or to pyruvate in muscle. Thus, only the aforementioned five amino acids could theoretically supply the carbon skeleton for alanine synthesis. Garber et al (47) and Goldstein et al (48) reported that aspartate, cysteine, leucine, valine, methionine, isoleucine, serine, threonine, glycine all significantly increased the formation and release of alanine from muscle in vitro and consequently concluded that the carbon chains from these amino acids are used in alanine synthesis. However, these experiments were carried out with amino acid concentrations approximately 100 times physiologic levels. At physiologic concentrations, their effects appear to be insignificant (45, 49-51).

In order to conclusively settle the controversy regarding the origin of alanine's carbon skeleton, Chang et al (50) measured the rates of pyruvate generation from exogenous glucose and from rat muscle protein breakdown in the rat diaphragm. The rate of glyco-

lysis was measured by the generation of ^{3}HOH from exogenous 5-^{3}H-glucose. The rate of generation from protein breakdown of the five amino acids that enter the TCA cycle was estimated from the measured rate of tyrosine release and the relative proportion of tyrosine and the five amino acids in muscle. The authors concluded that, even if all the carbon skeletons from the five amino acids were to be converted to pyruvate, 97% of the carbons used for alanine synthesis in muscle must still derive from exogenous glucose rather than from endogenous protein catabolism. Finally, Ben Galim et al (52), in their recent in vivo tracer studies on dogs, found that the quantity of glucose extracted by the dog hindlimb could generate four times the amount of pyruvate necessary to account for the alanine produced, confirming the prominent role of glucose-derived pyruvate in alanine synthesis.

That BCAA are peripherally metabolized was first suggested by the studies of Miller (44) who noted that labelled valine, leucine, and isoleucine are catabolized at similar rates in normal and hepatectomized animals. Subsequently, Odessey and Goldberg, in their studies on diaphragms from fed rats using ^{14}C-labelled amino acids (14, 45), demonstrated that the catabolism of the BCAA to CO_2 proceeds at a rate comparable to their incorporation into muscle protein. By contrast, none of the other amino acids was significantly degraded to CO_2. Furthermore, in diaphragms from fasted rats, oxidation of BCAA increased its production of alanine and glutamine in a concentration-dependent manner. The addition of other amino acids, even at 2-5 times their normal concentrations, failed to stimulate alanine production.

Transamination of BCAA occurs almost exclusively with alpha-ketoglutarate to form glutamate which may either donate its amino group to pyruvate to form alanine or incorporate free ammonia to form glutamine (21, 51). In support of this are the following facts: transamination of BCAA with alpha-ketoglutarate occurs at a rate 100 times that with pyruvate, making the direct transamination with pyruvate to form alanine unlikely (21); the intramusccular concentration of alpha-ketoglutarate is close to the K_m of alpha-ketoglutarate transaminase (21); BCAA increases the in vitro production of glutamate as well as alanine (21). Most recently, Ben Galim et al (52) studied BCAA nitrogen transfer to alanine in vivo in dogs by measuring the transfer of ^{15}N from leucine to alanine in the whole animal as well as across the isolated hindlimb. They found that nearly half of leucine nitrogen was transferred to alanine, and it represented 18% of the nitrogen in alanine. If the nitrogen contribution of the other BCAA, valine and isoleucine, is comparable, then greater than half of alanine nitrogen could be derived from BCAA. Interestingly, they found that each hindlimb could only account for 6% of the total body ^{15}N-alanine production. The authors postulate that nitrogen may be transferred from the peripherally degraded BCAA to a metabolic intermediate which then transfers it to alanine in other body tissues. The possibility that glutamine might serve as this intermediate is intriguing in view of its prominent conversion to alanine in the intestine.

A number of clinical observations have underscored the physiologic importance of the glucose-alanine cycle and have supported the

primacy of BCAA and glucose-derived pyruvate in the peripheral synthesis of alanine in vivo. Children with maple syrup urine disease (MSUD) have a branched chain alpha-ketoacid dicarboxylase deficiency which blocks BCAA catabolism (54, 55). These patients have plasma alanine levels 3-10 fold lower than normal and suffer from hypoglycemic episodes (21, 43), despite normal concentrations of glucoregulatory hormones (56). Recent studies by Haymond et al (56) have revealed that in the basal state, patients with MSUD have elevated plasma leucine and decreased alanine levels. After a 24 hour fast, their BCAA concentrations rise, in marked contrast to controls. In addition, basal alanine flux rates, measured using stable isotopes, were strikingly lower in MSUD patients than in controls. Finally, in one patient with a partial enzymatic deficiency, the plasma alanine and BCAA levels as well as the alanine flux rate were intermediate between those of controls and classic MSUD patients. These studies suggest that BCAA degradation is a critical and rate-limiting event for alanine synthesis in vivo. Children with ketotic hypoglycemia are also prone to hypoglycemic episodes after brief fasting (57, 58). These episodes are associated with lowered alanine and raised BCAA levels (57). That hepatic gluconeogenesis is unimpaired in these patients is strongly suggested by the fact that alanine infusion results in prompt hepatic glucose production and resolution of symptoms (54). The above observations suggest that this syndrome, too, may be due to impaired BCAA degradation.

The primacy of pyruvate as a precursor for peripheral alanine synthesis is suggested by observations in exercising man, in whom peripheral glucose consumption and arterial alanine levels rise in

direct proportion to elevations in arterial pyruvate concentrations (7, 23, 59). In McArdle's syndrome (23), a disorder characterized by myophosphorylase deficiency and impaired muscle glycogen utilization, the arterial pyruvate level falls after exercise, and this is accompanied by a decline in alanine concentration. Finally, in children with inborn errors of metabolism resulting in hyperpyruvic-acidemia, arterial alanine levels are elevated (60).

V. The Effect of Insulin and Glucagon on Amino Acid Metabolism

Apart from their regulation of gluconeogenesis (discussed above), insulin and glucagon play an important role in amino acid metabolism. Weichmann (61) first reported in 1924 that, after insulin administration, diabetics exhibit decreased levels of blood amino acid nitrogen. Numerous studies (62-65) in animals and humans have since confirmed the ability of both endogenous and exogenous insulin to lower amino acid concentration. Zinneman et al (62) and Felig et al (63) have reported significantly depressed arterial levels of valine, leucine, isoleucine, tyrosine, phenylalanine, methionine, following endogenous insulin secretion in man. That insulin lowered amino acid levels by a peripheral effect was first suggested by studies demonstrating a progressive rise in amino acid concentrations in cats after evisceration (64) - a rise prevented by insulin administration. Lotspeich (65) observed that dogs, after insulin administration, exhibited lowered blood amino acid levels in proportion to their relative concentrations in muscle. Subsequently, Pozefsky et al (3) infused physiologic quantities of insulin into the brachial artery of human subjects and measured amino acid balances across the forearm. They noted a 75% decline in total amino

acid nitrogen output, with a consistent decline for only leucine, isoleucine, threonine, phenylalanine, tyrosine, glycine. The fact that the very same amino acids whose concentrations declined after systemic insulinization (62, 63) exhibited diminished forearm release after local insulinization suggested that insulin's systemic effect is the result of its inhibitory action on muscle amino acid release. The above studies have also revealed that alanine arterial concentration and net muscle release are not significantly affected by insulin. This probably reflects insulin's ability to stimulate alanine synthesis via the glucose-alanine cycle (21, 23) and not alanine's insensitivity to the hormone.

In assessing insulin's effect on net amino acid muscle release, one must consider its effect on amino acid transport, protein synthesis, and protein degradation. Several in vitro studies (61, 66) have demonstrated that insulin, in fact, stimulates all three processes. Fulks et al (66) have documented in rat diaphragms that insulin's effects on protein synthesis and degradation are quantitatively comparable with regard to their contribution to the regulation of peripheral amino acid release. Jefferson et al (61) confirmed these findings in perfused rat hemicorpus and found that insulin deficiency reduced the rate of protein synthesis by blocking peptide chain initiation. Pain and Garlick (67) studied protein synthesis in skeletal muscle of normal, streptozotocin diabetic and insulin-treated rats in vivo, by constant infusion of labelled amino acids. They found that the diabetic rats exhibited a 70% reduction in their rate of protein synthesis which was not seen in insulin-treated rats.

Although its role in the regulation of hepatic gluconeogenesis has been fairly extensively studied (2, 5, 23, 26, 31, 39-43), glucagon's physiologic effects on extrasplanchnic amino acid metabolism remain somewhat obscure. Bondy and Cerdillo (68) first noted in 1956 that glucagon infusion resulted in a steady decline in whole blood alpha amino nitrogen concentration. The hypoaminoacidemic effect of glucagon has since been confirmed by numerous studies (73-75) and has been demonstrated to be most striking for alanine. Several in vitro and in vivo studies have suggested that glucagon has a catabolic extrahepatic effect on amino acid metabolism (70-74). Peterson et al (70) first reported that glucagon increases amino nitrogen release by rat adductor muscle in vitro. Hait et al (71) have shown that glucagon inhibits incorporation of amino acids into myocardial protein. Buse et al (72) demonstrated that glucagon stimulates BCAA oxidation in isolated rat myocardium. The physiologic significance of the aforementioned studies (68-72) is uncertain inasmuch as they all employed pharmacologic doses of glucagon. In addition, since glucagon causes insulin secretion (2, 73) which, in turn, influences amino acid metabolism, the independent effect of glucagon cannot be deduced from the above studies.

To examine the independent effect of glucagon on extrasplanchnic amino acid metabolism, Lacy et al (73) infused glucagon into insulin-dependent diabetics and found that alanine's arterial concentration fell dramatically without a significant change in its splanchnic uptake, suggesting a peripheral effect of glucagon. They infused supraphysiologic doses of glucagon, however. Marliss et al (74) continuously infused insulin-deficient fasting human sub-

jects with physiologic doses of glucagon. They observed a 15% reduction in total amino acid concentration and, in the absence of a rise in urea excretion, postulated an extrasplanchnic effect of glucagon on amino acid metabolism. Pozefsky et al (75) subsequently directly studied glucagon's effect on peripheral amino acid metabolism by infusing physiologic doses of glucagon into the brachial artery of postabsorptive and insulin-deficient starved human subjects. They found that, in both postabsorptive and starved states, physiologic increments of glucagon have no effect on amino acid balance across forearm tissue in man.

VI. Intestinal Absorption of Amino Acids

The major products of intraluminal protein digestion are amino acids and small peptides, each of which is absorbed across the intestinal mucosa via an independent transport mechanism. Studies in animals and man (77-80) have demonstrated the presence of and characterized four distinct amino acid transport systems - for neutral amino acids (which include the BCAA, alanine, glycine, glutamine, methionine, phenylalanine, typtophan, tyrosine), dibasic amino acids, dicarboxylic (acidic) amino acids, and imino acids plus glycine. The neutral amino acid transport system, the most functionally important and extensively studied mechanism, is an active process, transporting amino acids uphill against a cell to lumen concentration gradient; is sodium dependent; and involves the formation of a ternary complex comprising sodium, amino acid, and a membrane carrier (77-79). It has also been demonstrated that there is competition for transport sites amongst amino acids sharing a common transport mechanism (81). Thus, the rates at which individual amino

acids are absorbed are significantly altered by the presence of other amino acids. Studies in animals (86, 83) and man (81) have demonstrated that the BCAA and methionine have the highest affinity for the neutral transport system and are therefore absorbed most rapidly. In addition, these amino acids inhibit the absorption of amino acids with lower transport affinities such as threonine and typtophan.

Although early studies suggested that there is significant intraluminal mixing of exogenous and endogenous protein after a meal, Adibi et al (84) conclusively demonstrated that in man, after a protein meal, there is a strong correlation among the increase in intraluminal amino acid concentrations, the magnitude of rise in plasma amino acid levels and the amino acid composition of the ingested protein. The authors concluded that exogenous protein constitutes the primary component of the intraluminal amino acid rise after a protein meal. They found, furthermore, from the amino acid analysis of ileal and jejunal aspirates after protein ingestion, that the process of protein digestion extends for greater than four hours and occurs in the ileal as well as the jejunal segments of the small intestine. Several studies in animals (9, 19) and man (20, 76) have since confirmed that the combined processes of digestion and absorption take at least 3-4 hours.

VII. Influence of the Route of Amino Acid Administration on its Metabolism

It is known that oral glucose administration results in a greater insulin secretory response than does equivalent intravenous administration (85, 86). It has furthermore been demonstrated that

oral glucose stimulates the secretion of the gastrointestinal hormone, GIP (gastric inhibitory polypeptide), which in turn enhances the release of insulin from pancreatic beta cells (87). It was thought that the hyperinsulinemia associated with oral glucose ingestion accounted for the superiority of oral glucose tolerance. Recently, however, Lickley et al (88) and DeFronzo et al (103) in the dog and human, respectively, have demonstrated that the augmented hepatic glucose extraction observed following glucose ingestion as compared to intravenous administration is not the result of concomitant hyperinsulinemia or hyperglycemia but reflects the stimulation by oral glucose of a gastrointestinal factor, as yet unidentified, which enhances insulin's effect on hepatic glucose metabolism.

The role of the splanchnic bed in the metabolism of intravenously administered amino acids has not been examined. Furthermore, despite the widespread use of amino acid solutions in parenteral alimentation, there is little data on the influence of the route of amino acid administration on its metabolism. Bypassing the gut and liver, intravenously administered amino acids might escape significant hepatic extraction and more effectively replete muscle nitrogen then ingested amino acids (20, 76). In addition, if after ingestion of amino acids, as after glucose, a gut factor were to be secreted which modulated insulin's effect on hepatic amino acid metabolism, then its absence following intravenous amino acid administration might favor the espace of amino acids to the periphery to replete muscle nitrogen. In this regard, Bloomgarden et al (19), in their recent studies on amino acid metabolism by the liver and intestine after protein and glucose ingestion, found a strong

correlation between gut output and hepatic uptake of amino acids. They postulated the existence of a mechanism, the mediators of which are unknown, which tightly links amino acid release by intestinal tissues with uptake by hepatic tissues. Their findings suggest that the route of amino acid administration is an important determinant of its hepatic extraction. Waterhouse et al (89) examined the change in amino acid arterial concentration and arteriovenous difference across the forearm of human subjects undergoing parenteral alimentation with amino acid solutions. They found that, despite up to five-fold increases in amino acid arterial levels, there was no significant positive change in the arteriovenous difference across the forearm for any of the administered amino acids. They suggested that the initial processing of amino acids during repletion of muscle nitrogen occurs in extraskeletal tissues. One of the objectives of the current study was to elucidate the roles of the hepatic and intestinal tissues in the metabolism of amino acids administered via the intravenous route as well as to examine the influence of the route of administration on amino acid metabolism.

VIII. Amino Acid Transport

Since Winter et al (90) reported that the migration of amino acids across the human erythrocyte membrane occurs at a very slow rate in vitro, it has been generally accepted that plasma rather than erythrocytes is the primary vehicle for interorgan amino acid transport. The validity of this assumption was first questioned by Elwyn et al (25, 9) whose in vivo dog studies revealed that red blood cells do indeed play a significant role in amino acid transport. They demonstrated that the rates of change of concentration

and specific activity of glycine and serine in erythrocytes during transversal of the capillary and sinusoidal beds of gut and liver were twenty-fold faster than rates observed between plasma and erythrocytes in vitro. They concluded that there is direct exchange of amino acids between tissue cells and erythrocytes as the latter pass through gastrointestinal organs and that this exchange is independent of and frequently progresses in the opposite direction of that between plasma and tissues cells.

The importance of red blood cells in interorgan amino acid transport in man was first suggested by Aoki et al (91) who found that the stimulatory effect of insulin on muscle glutamate uptake was only manifested upon analysis of whole blood but not plasma arterio-deep venous differences. They thus implicated circulating erythrocytes as an important vehicle for glutamate transport and underscored the need for whole blood analysis in investigations of interorgan amino acid movements. Felig et al (8) subsequently determined peripheral, splanchnic and portal exchange of seventeen individual amino acids in healthy postabsorptive human subjects, measuring both plasma and whole blood arteriovenous differences. They found that blood cellular elements carried significant quantities of all amino acids in a direction which generally paralleled the net fluxes occurring in plasma. Glutamine was unique in that its movement via plasma and erythrocytes proceeded in opposite directions upon passage through the intestine. Alanine predominated in amino acid transport via erythrocytes as it does via plasma, with 30% of its leg and intestinal output and 22% of its splanchnic uptake occurring by the former mode of transport. Finally, Aoki et al

(22) measured amino acid differences after protein ingestion across the forearm of a single nitrogen-depleted subject fasted for six weeks. They found that one-third to one-half of peripheral BCAA uptake occurred via erythrocytes. As a result of the above data documenting the pivotal role of red blood cells in interorgan amino acid transport, all amino acid measurements in the current study were performed on whole blood samples.

METHODS

Subjects and Materials. Unanesthesized mongrel dogs of either sex weighing between 16.4 and 29.1 kgs (mean 22.7±1.6) were studied. (3-^{3}H) glucose from New England Nuclear (Boston, Mass.) served as the labelled tracer for glucose production. Glucagon and insulin were purchased from Eli Lilly Co. (Indianapolis, Ind.). FreAmine II 8.5% (composition [mg/dl]): isoleucine, 590; leucine, 770; lysine, 370; methionine, 450; phenylalanine, 480; threonine, 340; tryptophan, 130; valine, 560; alanine, 600; arginine, 310; histidine, 240; proline, 950; serine, 500; glycine, 1700; cysteine HCl, <20 was obtained from Abbott Laboratories (North Chicago, Illinois). Silastic catheters were purchased from Dow Corning Co. (Midland, Mich.). Hepatic blood flow was determined using Cardiogreen dye, Hynson, Westcott, and Dunning Co. (Baltimore, Md.). Infusions were carried out on Harvard Apparatus Infusion Pumps. Ion-exchange chromatography was performed on a Beckman Model 120C amino acid analyzer (Beckman Instrument Co., Palo Alto, Cal.). Plasma glucose was measured on a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, Oh.).

Surgical Procedure. Surgery was performed under halothane

anaesthesia via a midline laparotomy. Silastic catheters (.125" O.D., .062" I.D.) were inserted into the left common hepatic vein, portal vein, mesenteric vein, and a branch of the iliac artery. The left common hepatic vein was cannulated in antegrade fashion as described by Shoemaker et al (92). The tip of the catheter was modified by the deposition of two raised silastic rings between which a purse string suture was placed, securing the catheter in its place and preventing its advancement into the inferior vena cava. The portal catheter was guided cephalad through a tributary of the portal vein until its sampling tip rested at the porta hepaticus, allowing for the sampling of mixed portal blood including pancreatic drainage. Catheters were flushed early with saline to prevent clotting. At the end of the procedure, catheters were filled with heparin (300 U/ml) and brought out through the abdominal fascia and then tunneled subcutaneously to the midscapular region, occluded, and buried in a subcutaneous pouch. An incision, through which the catheters were drawn to the outside at the time of experimentation, was sutured to prevent the dog from gaining access to the catheters and to lower the risk of infection. One to three weeks were allowed for recovery from surgery, during which time dogs were maintained on a diet comprising 450 g of canned meal daily and unlimited dry chow.

Experimental Design. After an overnight 18 hour fast, dogs were restrained in harnesses. Skin in the midscapular region was infiltrated with lidocaine, catheter ends were exteriorized and their patency maintained by a continuous saline drip. A 16-gauge intravenous catheter was placed in the saphenous vein for the infu-

sion of indocyanine green dye, amino acids, and 3-^{3}H-glucose. Primed (3 mg) continuous (300 ug/min) infusions of indocyanine green dye were begun 90 min prior to and continued for the duration of the studies. Body glucose turnover was measured in 4 dogs in both the intravenous and oral studies. Dogs received primed (15 µCi) continuous (0.15 µCi/min) infusions of 3-^{3}H-glucose 90 min prior to and for the length of the studies.

In the oral studies, nine dogs consumed within 5 minutes 700 mg/kg body weight of the crystalline amino acid mixture Freamine II$^{(R)}$ 8.5%, diluted 1:1 with isotonic saline. Dogs in the intravenous studies received a 10 minute priming dose followed by a continuous infusion of Freamine II$^{(R)}$ 8.5%. Five dogs received a total dose of 720 mg/kg (low-dose infusion) and five received a total of 1440 mg/kg (high-dose infusion) of amino acids. The rates of infusion of 14 amino acids during the last 170 minutes of the low-dose studies are listed in Table 10. In all I.V. studies, the rate of infusion of the priming dose was twice that of the continuous infusion. The rate of infusion in the low-dose studies was calculated to achieve whole blood amino acid levels similar to those obtained in the oral studies in order to assess the influence of the route of administration on amino acid disposal independent of differences in concentration. In two of the dogs receiving low-dose infusions the route of administration was via a mesenteric rather than a saphenous vein. This was undertaken to further elucidate the effect of the route of administration on hepatic amino acid metabolism.

In all studies, whole blood samples for determination of amino acid concentrations were collected simultaneously from the hepatic vein, portal vein, and a branch of the iliac artery at timed inter-

vals during a 30 minute basal period and for 3 hours after Freamine$^{(R)}$ administration and set on ice. Plasma glucose concentration, 3-^{3}H-glucose specific activity, and arterial blood hematocrit were determined at 15 minute intervals for 30 minutes prior to amino acid administration and for the duration of the studies. Portal plasma glucagon and portal and arterial insulin concentrations were determined twice in the postabsorptive state and at 30 minute intervals throughout the studies.

Calculations. Hepatic plasma flow was determined according to the Fick principle by a continuous infusion of indocyanine green dye according to the methods described by Bradley et al (93) and Leevy et al (94). Hepatic blood flow was calculated by dividing the plasma flow by (1 - hematocrit). Indocyanine green dye was determined spectrophotometrically at 805 nm in serum samples. Uptake and production of metabolites in the splanchnic area were calculated by the arteriovenous (A-V) difference technique. This was accomplished by determining arterial, portal and hepatic venous metabolite concentrations and assuming that 80% of the hepatic blood flow is derived from the portal vein while 20% comes from the hepatic artery (95). The actual formulae used in the determination of the hepatic, splanchnic and extrahepatic splanchnic organ balances were as follows:

Splanchnic Substrate Balance =

$$([\text{Substrate}]_A - [\text{Substrate}]_{HV}) \times \text{Hepatic Flow}$$

Extrahepatic Splanchnic Substrate Balance =

$$([\text{Substrate}]_A - [\text{Substrate}]_{PV}) \times 0.8 \times \text{Hepatic Flow}$$

Hepatic Substrate Balance =

$$\left[([\text{Substrate}]_{PV} - [\text{Substrate}]_{HV}) \times 0.8 + ([\text{Substrate}]_{A} - [\text{Substrate}]_{HV}) \times 0.2\right] \times \text{Hepatic Blood Flow}$$

where subscripts A, HV, and PV designate artery, hepatic vein, and portal vein, respectively.

From the above formulae, it is apparent that:

Splanchnic Substrate Balance =

Hepatic Substrate Balance + Extrahepatic Splanchnic Substrate Balance

The hepatic extraction ratio was calculated using the following equation:

Hepatic Substrate Extraction Ratio =

$$\frac{(0.8 \times [\text{Substrate}]_{PV} + 0.2 \times [\text{Substrate}]_{A}) - [\text{Substrate}]_{HV}}{(0.8 \times [\text{Substrate}]_{PV} + 0.2 \times [\text{Substrate}]_{A})}$$

While amino acid balances were determined using whole blood concentrations and hepatic blood flow, glucose balances were calculated employing plasma levels and hepatic plasma flow inasmuch as erythrocytes do not actively participate in interorgan glucose transport in the dog (95).

In addition to the A-V difference technique, the rates of endogenous glucose production and utilization following amino acid administration were determined employing the primed tracer infusion method. Calculation of the rates of appearance (Ra) and disappearance (Rd) of glucose was performed according to the two-compartment analysis of glucose kinetics proposed by Radziuk et al (96). This model corrects for the fact that rapid changes in the concentration and specific activity of glucose do not occur uniformly

throughout the glucose pool. It assumes that the first compartment contains the vascular space, and that it is this compartment in which concentrations of labelled and unlabelled glucose are determined and glucose and tracer appear. In this manner, reliable measurements of non-steady state turnover rates have been attained. The metabolic clearance rate of glucose was calculated from the ratio of Rd to plasma glucose.

To determine 3-^3H-glucose, plasma was deproteinized using zinc sulfate, and barium hydroxide as the alkali according to the Somogyi-Nelson technique (97, 98). The protein-free supernatant was placed in a vacuum oven at 70°C overnight in order to evaporate radioactive water from the glucose samples. Samples were then reconstituted with water and their radioactivity determined using liquid scintillation procedures.

All amino acid determinations were performed on whole blood. Blood for amino acid analysis was deproteinized with equal volumes of 10% sulfosalicylic acid (99) and spun at 10,000 r.p.m. for 20 minutes. To remove glutathione, which emerges as a broad peak underlying many amino acids and thus interfers with the chromatographic determination of amino acids in whole blood, the whole blood filtrates were treated with sodium sulfite prior to chromatographic analysis. The filtrates (2.0 ml) were first neutralized to pH 7.0 with 4N NaOH, after which (0.1 ml) 0.5M sodium sulfite was added and the solution placed in a shaker at room temperature for two hours. Filtrates were then reacidified with HCl to pH 2.2 (99) and determinations made using automated ion-exchange chromatographic techniques (99) on a Beckman Model 120C amino acid analyzer.

Plasma glucose was analyzed by the glucose oxidase reaction (100) using a glucose analyzer. Plasma glucagon was analyzed by radioimmunoassay using Unger antibody 30K (102). Insulin was determined with talc to separate bound from free insulin. Hematocrit was measured for each time interval using a microcapillary hematocrit centrifuge.

Data in the text, tables, and figures are given as the mean ± SEM. Standard statistical methods have been employed (102), with paired t-test when applicable.

RESULTS

I. Oral Studies

A. Postabsorptive State

a. Whole Blood Amino Acid Concentration and Balances. The whole blood arterial, portal, and hepatic venous concentrations of ten amino acids in nine dogs in the postabsorptive state are shown in Table 1. Postabsorptive hepatic, extrahepatic splanchnic, and splanchnic balances for these amino acids (Table II) are in close agreement with earlier observations (2, 6-9, 19). In the basal state, the extrahepatic splanchnic tissues produced significant amounts of alanine, but net extrahepatic splanchnic balances were not significant for any of the other glucogenic or branched chain amino acids. The liver removed alanine and other glucogenic amino acids but not the BCAA. The basal hepatic extraction ratios for alanine, glycine, and serine were 31±5%, 22±4%, and 29±4%, respectively, while those for the BCAA were not significantly different from zero (Table III). There was a net splanchnic uptake of alanine, reflecting the hepatic metabolism of alanine derived from both extrahepatic splanchnic and peripheral tissues.

b. Plasma Glucose Concentrations and Balances. The mean basal arterial, portal, and hepatic venous concentratons were 97±3, 95±4, and 105±3 mg/dl, respectively, reflecting hepatic production and intestinal extraction of glucose in the postabsorptive state. The rates of extrahepatic splanchnic removal, hepatic production, and net splanchnic production of glucose were 0.8±0.1, 2.9±0.3, and 2.1±0.3 mg/min•kg, respectively.

B. Postcibal (i.e., Post-Ingestion) State

a. Whole Blood Amino Acid Concentrations and Balances. The whole blood arterial, portal and hepatic venous concentrations and the hepatic, extrahepatic splanchnic, and splanchnic balances for ten amino acids during the 3 hours post-ingestion are shown in Tables I and II, respectively. The portal concentrations of all amino acids present in the Freamine II$^{(R)}$ mixture rose by 15 minutes post-ingestion, reflecting prompt intestinal uptake. Peak portal concentrations (Table I) and extrahepatic splanchnic release (Table II) of ingested amino acids generally occurred at 60-90 minutes post-feeding, although intestinal absorption, as reflected by increased portal concentrations and extrahepatic splanchnic release, was still ongoing at 180 minutes. The mean hepatic, extrahepatic splanchnic, and splanchnic balances for alanine, glycine, proline, serine, and tyrosine are shown in Figure I. Those for leucine, isoleucine, and valine are shown in Figure II. For all amino acids, intestinal absorption was accompanied by an increase in hepatic uptake. For glycine, proline, and serine, mean hepatic uptake generally paralleled mean extrahepatic splanchnic release, resulting in a net splanchnic balance similar to that in the postabsorptive state. For alanine, hepatic uptake exceeded extrahepatic splanch-

nic output, resulting in an increased net splanchnic uptake above that in the postabsorptive state. Intestinal output of BCAA exceeded their extraction by the liver, resulting in a net splanchnic release of these amino acids.

The amino acid doses as well as the cumulative hepatic, extrahepatic, and splanchnic balances during the 3 hr postcibal period are shown in Table IV. On comparing the extrahepatic splanchnic balances and the oral doses of each amino acid, it is apparent that all of the glucogenic amino acids and 80% of the BCAA were absorbed during the 3 hr study. The cumulative intestinal release of alanine exceeded the administered dose by 47%, reflecting continued gut production of this amino acid in the postcibal state. In addition, during the 3 hr postcibal period, the liver removed 1736±166 μmoles/kg of alanine, a quantity three-fold greater than the oral dose of alanine and two-fold greater than its extrahepatic splanchnic output which comprises both the ingested load and the postcibal intestinal production of alanine. The rate of splanchnic alanine uptake was 63% greater than that in the basal state. In association with alanine's rising arterial level in the postcibal state, this suggests stimulation of peripheral alanine production in the fed state.

The liver took up 44% of the absorbed and 35% of the administered load of BCAA. However, since the extrahepatic splanchnic release exceeded their removal by the liver, there was a net splanchnic release of these amino acids to the periphery. Over the 3 hrs following protein ingestion, a total of 538±183 μmoles/kg of BCAA were released from the splanchnic bed. During this same time period, there was a net splanchnic uptake of 1480±450 μmoles/kg of glucogenic amino acids, of

which alanine constituted 891±142 μmoles/kg (Table IV). Thus, despite the splanchnic escape of BCAA, the net flux of amino acids from the peripheral to the splanchnic tissues persisted in the fed state. Finally, the hepatic extraction ratios of all amino acids rose after protein ingestion although only the increases in the extraction of glycine, proline, leucine, and valine were statistically significant (i.e., $p<0.05$) (Table III).

b. Plasma Glucose Concentrations and Balances. The mean plasma arterial, portal, and hepatic venous concentrations of glucose in nine dogs in the postabsorptive state and for the 3 hrs following amino acid ingestion are plotted in Figure 3. Glucose concentrations in all three vessels fell slightly over the duration of the study and by 180 min were 7 mg/dl below basal. The hepatic, extrahepatic splanchnic, and splanchnic glucose balances in the basal state and for 3 hours post-ingestion are shown in Figure 4. Intestinal uptake of glucose rose shortly after ingestion, peaked at 75-120 mins, and returned to basal by 165 mins. The rate of hepatic glucose release rose from 2.87±0.3 mg/min•kg in the postabsorptive state to 4.15±0.2 mg/min•kg at 75-90 mins and returned to baseline by 150 mins. Net splanchnic glucose release rose from 2.10±0.3 mg/min•kg to a peak of 2.99±0.1 by 75-90 min and returned to basal by 150 min. Inasmuch as the extrahepatic splanchnic tissues took up glucose throughout the postcibal period, the rise in the splanchnic release of glucose was solely due to its increased hepatic production.

The rates of appearance (Ra), disappearance (Rd) and metabolic clearance of glucose in four dogs in the basal and postcibal states are shown in Figure 5. All three parameters rose shortly after protein ingestion and peaked at 45-60 min. The rise in Rd exceeded that of Ra, accounting for the slight decline in arterial glucose concentration. By 105 min

after ingestion, all three parameters had returned to their basal values.

Mean portal glucagon and insulin levels in the basal and post-cibal states are shown in Figure 6. The mean portal glucagon concentration rose immediately following ingestion, peaked at 2½ times basal by 30 min, and remained significantly elevated ($p<0.05$) for the entire 3 hr post-cibal period. Mean portal and arterial insulin levels rose significantly ($p< 0.050$) by 30 min post-ingestion but returned to baseline by 90 min.

Hepatic plasma flow was 37±3 ml/min•kg in the postabsorptive state and did not significantly change during the 3 hr post-cibal period (Figure 3).

II. Intravenous Studies

A. Postabsorptive State

a. Whole Blood Amino Acid Concentrations and Balances. The mean whole blood arterial, portal, and hepatic venous concentrations in the postabsorptive state in 5 dogs receiving low dose infusions and 5 dogs receiving high dose infusions are shown in Tables 5 and 6, respectively. Hepatic, extrahepatic splanchnic, and splanchnic balances in the basal state and during high-dose I.V. amino acid infusion are shown in Figures 7 and 8. Basal intestinal and hepatic metabolism of BCAA and glucogenic amino acids was similar to that observed in the oral studies. For the BCAA, there was no significant uptake or release by either the intestine or the liver. Alanine was released by the gut, removed by the liver, and taken up by the splanchnic bed as a whole in significant quantities. The postabsorptive hepatic extraction ratios for alanine, glycine, and serine were 35±6, 27±4, and 24±6, respectively, while those for the BCAA were not significantly from zero (Table 7).

b. Plasma Glucose Concentrations and Balances. In the 5 dogs who

received high-dose infusions, mean plasma arterial, portal and hepatic venous concentrations in the postabsorptive state were 99±3, 97±3, and 107±3, respectively (Figure 9). In the 5 dogs receiving low dose infusions, arterial, portal and hepatic venous concentrations were 98±5, 96±5, and 105±5, respectively. The rates of extrahepatic splanchnic uptake, hepatic production, and net splanchnic production of glucose in the low dose group were 0.6±0.2, 2.9±0.6, and 2.3±0.5, respectively (Figure 10). In the high-dose group, the corresponding values were 0.6±0.1, 2.6±0.3, and 2.0±0.3 (Figure 9). In the 4 dogs whose glucose turnover was measured isotopically, postabsorptive rates of glucose appearance (Ra), disappearance (Rd), and metabolic clearance (MCR) were each 3.8±0.2 mg/min·kg (Figure 11).

B. Intravenous Amino Acid Infusion

a. Whole Blood Amino Acid Concentrations and Balances.

1. High-Dose Studies. The mean whole blood arterial, hepatic and portal venous concentrations for ten amino acids during the 3 hr high-dose intravenous amino acid infusion are shown in Table 6. All amino acid concentrations peaked at 15 min, shortly after the infusion of the priming dose, delivered at a rate double that administered for the duration of the study. Glycine, the most abundant component of the Freamine II$^{(R)}$ solution, rose most prominently. Relatively steady blood levels in all 3 vessels were maintained during the last 2 hr of the study by the continuous infusion of the amino acid mixture. Arterial levels of the BCAA generally exceeded portal levels ($p<0.05$, for each) during the 3 hr infusion. As in the postabsorptive state, portal concentrations of alanine significantly ($p<0.001$) exceeded arterial levels at all times during the 3 hr infusion. In contrast, arterial and portal concentra-

tions of threonine, serine, proline, glycine, and phenylalanine were not significantly different from those in the postabsorptive state.

Analysis of hepatic, extrahepatic splanchnic, and splanchnic balances for the BCAA (Figure 6) reveals that during the 3 hr high dose infusion, hepatic, intestinal and net splanchnic uptake rose dramatically. All three parameters for valine, leucine, and isoleucine generally peaked by 15 min and remained elevated for the duration of the study. The contribution of the liver and the extrahepatic splanchnic tissues to the handling of the infused amino acid load, integrated over the 3 hr infusion period, is shown in Table 8. The hepatic and intestinal tissues both disposed of comparable quantities of BCAA and together metabolized 973±253 μmoles/kg or 37% of the administered BCAA load. Amino acid hepatic extraction ratios during the last 2 hr of the high-dose infusion, during which time blood amino acid levels were relatively steady, are shown in Table 7. Extraction ratios for leucine, isoleucine, and BCAA as a group rose significantly ($p<0.05$) from basal values.

During the high-dose infusion, intestinal handling of alanine and other glucogenic amino acids was not significantly altered from basal despite greatly elevated arterial levels of these amino acids. Alanine and glycine continued to be released by the extrahepatic splanchnic tissues at rates not significantly different from those in the postabsorptive state. By contrast, hepatic uptake and, in turn, net splanchnic removal of these amino acids were dramatically increased during the high-dose infusion ($p<0.001$). Rates of hepatic and net splanchnic uptake of alanine and the other glucogenic amino acids generally peaked by 30 min and remained significantly elevated by 180 min. The cumulative splanchnic uptakes, i.e., over the entire 3 hr infusion, of alanine, glycine,

and serine equalled or exceeded the total administered doses of these amino acids (Table 8). Despite this, arterial concentrations of these amino acids were continually elevated, reflecting continued net peripheral production of these amino acids during the infusion. For phenylalanine and proline, net splanchnic removal accounted for 75% and 53%, respectively, of the total administered dose of these amino acids. Of the 58 μmoles/kg·min of amino acids infused over the 3 hr study, 51 μmoles/kg·min were metabolized by splanchnic tissues and 7 μmoles/kg·min at peripheral sites (Table 8). The hepatic extraction ratios for alanine, glycine, serine, and proline all rose during the infusion, but only the increase in the hepatic extraction ratio for proline was statistically significant (i.e., $p<0.05$).

2. Low-Dose Studies. The rates of continuous infusion of 14 amino acids during the low-dose studies are shown in table 9. The mean whole blood arterial, portal, and hepatic venous concentrations for 8 amino acids during the 3 hr low-dose intravenous infusions are shown in Table 5. Amino acid concentrations in all three vessels rose dramatically by 15 min after the beginning of the infusion and remained relatively steady for the entire 3 hr study. Blood amino acid levels during the infusion were similar to those observed in the oral studies following amino acid ingestion.

Hepatic balances for 7 amino acids and the BCAA as a group are shown in Figure 11. Since 2 of the 5 dogs received low-dose infusions via a mesenteric vein, extrahepatic splanchnic and net splanchnic balances were not quantitated. Hepatic uptake of all amino acids rose shortly after the beginning of the infusion, generally peaked by 30 min, and remained elevated for the duration of the study. Cumulative hepatic amino acid

uptake for 5 dogs during the low-dose infusions is shown in Table 10. Hepatic removal of all glucogenic amino acids equalled or exceeded the administered dose of these amino acids. In association with continually elevated arterial levels of glucogenic amino acids, the results indicate that, as during high-dose amino acid infusion and following oral administration of amino acids, peripheral production of these amino acids continues during the low-dose infusions. The liver extracted 39±5%, 28±4%, and 40±6% of the administered doses of leucine, isoleucine, and valine, respectively.

The hepatic extraction ratios of all amino acids rose during the infusions, but none of the increases reached statistical significance. The mean hepatic extraction ratios for total BCAA in dogs receiving peripheral or mesenteric low-dose infusions were 7±1% and 9±1%, respectively. Corresponding values for glucogenic amino acids as a group were 32±2% and 31±2%.

b. Plasma Glucose Concentrations and Balances.

1. High-Dose Studies. Plasma glucose arterial, hepatic, and portal venous concentrations all declined during the infusion, and by 180 min the arterial plasma glucose concentration was 12 mg/dl below basal (Figure 9). Hepatic glucose production rose from 2.6±0.3 mg/min•kg to a peak of 4.9±0.6 mg/min•kg at 15-30 min and remained significantly elevated (i.e., $p<0.05$) up to 135 min (Figure 10). Extrahepatic splanchnic as well as net splanchnic production of glucose also rose significantly (i.e., $p<0.05$) within minutes after the start of the infusion and remained significantly elevated to 75 and 135 min, respectively.

In the four dogs whose glucose turnover was measured isotopically the rate of glucose disappearance (Rd) rose significantly ($p<0.05$) from 3.8 mg/min•kg (basal) to 5.1±0.5 mg/min•kg, as did the metabolic clearance of glucose from 3.8±0.2 mg/min•kg to 5.4±0.5 mg/min•kg, both within

60 min after the beginning of the infusion. The rate of glucose appearance (Ra) rose as well but not to a statistically significant degree (Figure 11), thereby accounting for the concomitant fall in plasma arterial glucose concentration.

The mean portal plasma glucagon level rose from 162±40 pg/ml (basal) to 540±110 pg/ml by 30 min and remained at least three-fold elevated for the duration of the study (Table 11). Mean portal plasma insulin levels rose from 35±3 μU/ml (basal) to a peak of 121±19 μU/ml by 30 min, declined to 54±14 μU/ml by 60 min, and remained elevated for the duration of the study (Table 11). Arterial plasma insulin levels (not shown) similarly rose dramatically from 19±3 μU/ml (basal) to 63±5 μU/ml by 15 min, then fell to 26±3 by 60 min and remained elevated for the entire 180 min.

2. Low-Dose Studies. Plasma glucose arterial hepatic and portal venous concentrations all declined during the low-dose infusion (Figure 9). By 180 min, the arterial plasma glucose concentration was 8 mg/dl below basal. Hepatic, extrahepatic splanchnic, and splanchnic glucose balances were not significantly altered at any time during the low-dose infusion (Figure 10).

The mean portal plasma glucagon level rose from 217±78 pg/ml (basal) to a peak of 410±155 by 90 min and remained elevated for the remainder of the 3 hour study period. The mean portal insulin level rose as well from 35±5 μU/ml (basal) to a peak of 60±18 μU/ml by 90 min but returned to the basal level by 150 min.

Hepatic plasma flow did not significantly change during either the low-dose or high-dose infusions (Figure 9).

DISCUSSION

I. Oral Studies

The current studies demonstrate, in keeping with earlier observations (2, 7-9, 19), that in the postabsorptive state, the peripheral as well as the intestinal tissues produce significant amounts of alanine, all of which is effectively metabolized by the liver. There was no net exchange of other glucogenic amino acids across the extrahepatic splanchnic tissues in the basal state, but there was significant hepatic uptake of these amino acids, resulting in a small net splanchnic uptake. There was no significant net exchange of BCAA by either the liver or the extrahepatic splanchnic tissues. Overall, there was a net average flux of 6.2 μmoles/min•kg of amino acid nitrogen from the peripheral to the splanchnic tissues.

Following amino acid ingestion, portal concentrations and extrahepatic splanchnic release of all amino acids rose rapidly, reflecting prompt intestinal absorption. Over the 3 hr study, the entire ingested loads of serine, glycine, phenylalanine, and proline, as well as 80% of the ingested BCAA were recovered in the portal blood (Table 4). While the ingested amino acid mixture contained 580 μmoles/kg of alanine, 853 ±118 μmoles/kg were recovered over the 3 hr study, reflecting continued alanine synthesis by intestinal tissues in the fed state. Bloomgarden et al (19) reported similar findings in their recent studies in dogs. Following feeding, there was a mean net splanchnic alanine uptake of 4.95±0.8 μmoles/min•kg, a rate 63% greater than that observed in the postabsorptive state. In association with rising arterial concentrations of alanine, this indicates stimulation of peripheral alanine production in the post-cibal state and confirms the earlier observations of

Bloomgarden et al (19).

Although the origins of the carbon skeleton and amino group used in the peripheral synthesis of alanine were not investigated in the current studies, earlier observations (21, 23, 45, 49-52) strongly suggest that glucose-derived pyruvate and BCAA are the sources of the carbon skeleton and amino group, respectively (see Introduction). In this regard, net splanchnic release and peripheral uptake of glucose were significantly stimulated after amino acid ingestion (discussed below) to an extent which could provide sufficient carbon substrate for the peripheral alanine production observed. In addition, over the 3 hr study, 588±183 μmoles/kg of BCAA escaped the splanchnic bed to the periphery as potential substrate for alanine synthesis.

The likely source of the carbon skeleton used for intestinal alanine production in the post-cibal state is glucose-derived pyruvate as well. In this regard, intestinal uptake of glucose was significantly stimulated following amino acid ingestion (see below). Concerning the nitrogen source for intestinal alanine synthesis, the entire ingested load of all amino acids except the BCAA was quantitatively recovered in the portal blood, and it is thus unlikely that the former provided significant amounts of nitrogen substrate for alanine synthesis. Since the BCAA have the highest affinity for the neutral amino acid transport system (81-83), and are thus the most rapidly absorbed of all amino acids, it is unlikely that the lack of complete recovery in the portal blood of the ingested BCAA was due to impaired intestinal absorption. Rather, the 20% of BCAA not accounted for may have been used as substrate for intestinal alanine production. In addition, nitrogen may have been transferred from peripherally degraded BCAA to a metabolic intermediate such as glutamine, not measured in the current studies, which then trans-

ferred it to alanine in intestinal tissue (see Introduction). In support of this hypothesis are the tracer studies of Ben Galim et al (52), demonstrating that while nearly half of labelled leucine nitrogen was transferred to alanine, each hindlimb could account for only 6% of the total body labelled alanine production, suggesting that amino groups derived from the BCAA are transferred via an intermediate to alanine elsewhere in the body.

A striking finding in the present studies was the hepatic uptake of 35% of the ingested load of BCAA. Previous studies in dogs (9), rats (17), and sheep (18), have demonstrated very little hepatic removal of these amino acids. Bloomgarden et al, in their recent studies in dogs (19), noted a failure of the BCAA to be taken up by the liver. Thus, it has been generally assumed that the liver plays a passive role in the handling of these amino acids. The current studies conclusively demonstrate, however, that approximately 45% of the absorbed BCAA is removed by the liver and that the hepatic tissues thus play an important role in the metabolism of these amino acids. Whether a similar function is served by the liver in man remains to be resolved.

Analysis of the cumulative hepatic, extrahepatic splanchnic and splanchnic balances for all amino acids over the three postcibal hours (Table 4) reveals that the postabsorptive nitrogen flux from periphery to splanchnic tissues persisted after feeding. Net transfer of glucogenic amino acids from the skeletal muscle tissues to the splanchnic bed exceeded splanchnic escape of BCAA to the periphery by 895 moles/kg·3 hrs (Table 4). Similar observations

have been reported in dogs (19) and man (20, 76). In addition, glutamine, not measured in the current studies, has been shown both in sheep (18) and man (20) to be prominently released by skeletal muscle tissue in the fed state and metabolized by the intestinal tissues. Thus, the net flux of amino acids from the periphery to the splanchnic bed is likely greater than that measured in the present studies. BCAA alone, then, do not replete muscle nitrogen stores after a protein meal. Since it is known that in animals and man muscle protein stores are indeed maintained, some nitrogen substrate not measured in this and other studies (19, 20, 76) may complement the BCAA in their role as nitrogen carriers to the periphery.

Assessment of the hepatic extraction ratios in the postabsorptive and postcibal states (Table 3) reveals the liver's striking selectivity in its handling of amino acids. While the liver extracted 20-30% of the alanine, glycine, and serine presented to it in the basal state, it did not significantly extract any of the BCAA. Following ingestion, hepatic extraction of the BCAA rose to 6-9% and that of the glucogenic amino acids rose 6-13% above basal. Clearly, increased substrate delivery is one determinant of hepatic extraction. The influence of glucagon and insulin, both of which rose significantly after protein ingestion (see below), on hepatic amino acid extraction is uncertain. Although Brockman et al (41) have demonstrated a stimulatory effect of glucagon on hepatic alanine extraction in sheep, the studies of Jennings et al (26) in dogs and Chiasson et al (43) in man have demonstrated that glucagon exerts a purely intrahepatic stimulatory effect on gluconeogenesis but has no effect on alanine extraction. Felig et al (15) reported decreased splanchnic extraction of alanine in the presence of a rise in insulin

levels. Subsequent studies by Chiasson et al (36) in the dog and man (37), however, have demonstrated no effect by insulin on the hepatic extraction of alanine. The influence of the route of amino acid administration on hepatic extraction is addressed below.

Wahren et al (20) reported that after protein ingestion in man, net splanchnic production and arterial levels of glucose remain essentially unchanged. The current studies demonstrate that after protein ingestion in the dog, hepatic glucose production rises signficantly and remains elevated for 2½ hrs post-ingestion. Determinants of this rise in hepatic glucose production may include the increased delivery of glucogenic amino acids, with a resultant rise in hepatic gluconeogenesis, and the increased glucagon levels, with a resultant increase in either gluconeogenesis or glycogenolysis. Glucagon, portal concentrations of which rose to 2½-fold basal levels after protein ingestion, has been shown in dogs (26) to stimulate gluconeogenesis from alanine by regulating its intrahepatic metabolism. The precise fates of the various extracted precursors cannot be ascertained, however, without the concurrent use of tracer techniques, not employed in the present studies.

Although increments in arterial insulin levels comparable to those observed in the current studies have been shown to dramatically inhibit splanchnic glucose production (5), the concurrent hyperglucagonemia induced by protein ingestion resulted in a net increase in hepatic and net splanchnic glucose production. Results of the present glucose tracer studies reveal that peripheral glucose uptake, presumably insulin-mediated, exceeded the rise in hepatic glucose production and resulted in a slight fall in the arterial glcuose level. These results are consistent with the observation of Unger et al (29) that aminogenic hyperglucagonemia

serves to prevent a more prominent insulin-mediated decline in blood glucose after a carbohydrate-free protein meal by stimulating hepatic glucose production.

The current studies also demonstrate that intestinal uptake of glucose rises significantly and persistently after a protein meal. The extracted glucose may in turn furnish additional carbon substrate for the increased intestinal production of alanine in the post-cibal state.

Finally, the strong correlation observed between the quantities of individual amino acids ingested and those recovered in the portal blood confirm the earlier studies of Adibi et al (84) which demonstrated that there is little mixing of exogenous and intestinal endogenous protein after protein ingestion. Adibi et al (84) further demonstrated that the process of protein digestion exceeds 4 hrs. Several studies in animals (9, 19) and man (20, 76) have since confirmed that the combined processes of digestion and absorption exceed 4 hrs. Inasmuch as amino acid absorption was still ongoing at 180 min, the current studies, employing a free amino mixture, demonstrate that the absorptive process alone exceeds 3 hrs.

II. Intravenous Studies

The present studies clearly document a pivotal role for the splanchnic tissues in the metabolism of parenterally administered amino acids. During the high-dose amino acid infusion, there was no significant change in the handling of the glucogenic amino acids by the intestinal tissues. For the duration of the infusion, these tissues continued to produce alanine and glycine at rates similar to those observed in the postabsorptive state (Figure 8). Hepatic removal of these amino acids was dramatically increased, however (Figure 8). Over the 3 hr study, the liver removed

these amino acids in quantities which equalled or exceeded the administered doses (Table 8). Both the hepatic and intestinal tissues remove significant quantities of BCAA during the infusion. Of the 2638 μmoles/kg of BCAA administered, the hepatic and intestinal tissues removed 536±147 μmoles/kg and 436±162 μmoles/kg, respectively, and together accounted for the disposal of 37% of the infused load of these amino acids. Following amino acid ingestion, the liver alone removed 36% of the administered BCAA load.

During the 3 hr low-dose infusion, the liver similarly removed quantities of glucogenic amino acids equal to or greater than the administered doses of these amino acids. Despite this, during both the low-dose and high-dose infusion, arterial concentrations of the glucogenic amino acids rose, reflecting continued net peripheral production of these amino acids. The liver removed 36±4% of the total BCAA load infused over the 3 hr low-dose study.

Analysis of the cumulative hepatic, extrahepatic splanchnic, and splanchnic balances during the high-dose infusion (Table 8) reveals that over the 3 hr study, a total of 10,488 μmoles/kg of amino acids were infused while the splanchnic bed took up only 9229 μmoles/kg. Thus, 1259 μmoles/kg·3 hr or 7 μmoles/kg·min of amino acids (largely BCAA) were presumably metabolized by peripheral tissues. By comparison, after oral intake of the Freamine$^{(R)}$ mixture, there was a net flux of approximately 5 μmoles/kg·min from the peripheral to the splanchnic tissues. The inadequacy of the muscle nitrogen repletion provided by the BCAA during high-dose infusion is apparent if one recognizes that, in the postabsorptive state, there is an average flux of amino acid nitrogen of 6.2 μmoles/kg·min from peripheral to splanchnic tissues. During the 3 hr in-

fusion, then, the quantity of BCAA extracted by the peripheral tissues can replete nitrogen stores lost during only 3½ hrs of fasting. Thus, amino acids, whether administered orally or intravenously, do not adequately replenish muscle nitrogen. Some as yet unidentified nitrogen carrier, not measured in this and other studies (19, 20, 76), must then serve to maintain muscle nitrogen stores in the animal and man.

The hepatic extraction ratios for the BCAA and the glucogenic amino acids rose substantially during both the high-dose and low-dose infusions (Table 7), as they did following oral ingestion of amino acids. The mean hepatic extraction ratios for all amino acids were strikingly similar following oral, low-dose intravenous, and high-dose intravenous amino acid administrations (Figure 13). In addition, the mean hepatic extraction ratios for the peripherally and mesenterically administered low-dose infusions were strikingly similar. Thus, it appears that the quantity of substrate delivered rather than the route of administration is the major determinant of hepatic amino acid extraction. Bloomgarden et al (19) found a strong correlation between intestinal release and hepatic uptake of amino acids and postulated the existence of a mechanism, the mediators of which are not as yet identified, which tightly couples these two processes. The present studies militate against the existence of this link between hepatic and intestinal tissues, since oral and intravenously administered amino acids were extracted in nearly identical proportions. Although both insulin and glucagon rose during the low-dose infusions and quite strikingly during the high-dose infusions (Table 12), the influence of these increases in hormonal levels on hepatic amino acid extraction is questionable (see above). In addition, levels of both hormones were significantly higher during the high-dose infusions than during the low-dose infusions and following oral amino acid administration, yet

the mean hepatic extraction ratios in all 3 groups were nearly identical, arguing against the importance of hormonal levels as determinants of hepatic amino acid extraction.

The effect of parenterally administered amino acids on glucose metabolism was very similar to that observed following amino acid ingestion. As after amino acid ingestion, hepatic glucose production and net splanchnic glucose output rose following low-dose and high-dose amino acid infusions. The magnitude of the rise in hepatic glucose production was greater during the high-dose infusions than during the low-dose infusions and following ingestion of amino acids. As after oral administration, hihg-dose amino acid infusions resulted in a rise in the rates of appearance, disappearance and metabolic clearance of glucose (Figure 11). The rate of disappearance rose to a greater extent than the rate of appearance, resulting in a significant decline in plasma arterial glucose levels. Here, too, it is presumed that aminogenic hyperglucagonemia prevented a more precipitous drop in arterial glucose levels.

FIGURE LEGENDS

Figure 1: Mean balances of 5 glucogenic amino acids in the postabsorptive state (shaded bars) and for the three hour postcibal period (open bars) across the extrahepatic splanchnic (upper panel), hepatic (middle panel) and splanchnic (lower panel) areas. Each bar represents the mean ±1 SEM. Asterisks indicate significant differences between basal and postcibal values ***$P<0.01$.

Figure 2: Mean balances of leucine, isoleucine, valine, and combined branched chain amino acids in the basal state (shaded bars) and for the three hours following amino acid ingestion (open bars) across the extrahepatic splanchnic (upper panel), hepatic (middle panel) and splanchnic (lower panel) areas. Each bar represents the mean ±1 SEM. Asterisks refer to comparison of postabsorptive and postcibal values (*$P<0.05$, ***$P<0.01$).

Figure 3: Upper panel: Plasma glucose concentrations in the hepatic vein (H.V. -O-), artery (A -●-), and portal vein (P.V. -△-) in 9 dogs measured in the basal state and for 3 hours following amino acid ingestions. (error bars indicate 1 SEM.) Lower panel: Hepatic plasma flow during the basal state and following amino acid ingestion.

Figure 4: Extrahepatic splanchnic (upper panel), hepatic (middle panel), and splanchnic (lower panel) glucose balance in the postabsorptive state (broken line) and for 3 hours following amino acid ingestion. The broken line indicates the basal rates of glucose release or uptake. Asterisks indicate

values significantly different than basal ($*P<0.05$, $**P<0.02$). Error bars indicate 1 SEM.

Figure 5: The systemic rate of glucose appearance (upper panel), rate of disappearance of glucose (middle panel) and metabolic clearance rate (lower panel) of glucose in 4 dogs in the basal state (broken line) and after amino acid ingestion. Asterisk indicates values different than basal (P 0.05). Error bars indicate 1 SEM.

Figure 6: Concentrations of insulin and glucagon in portal plasma from 9 dogs (mean ±SEM) in the postabsorptive state and following amino acid ingestion. Values significantly different that postabsorptive $*P<0.04$, $**P<0.02$.

Figure 7: The mean (±SEM) extrahepatic splanchnic (upper panel), hepatic (middle panel) and splanchnic (lower panel) balances for each of three branched chain amino acids and for total BCAA in the postabsorptive state and during amino acid infusion. Significant differences between balances in the postabsorptive and amino acid infusion periods are indicated by asterisks)($0.05>P>0.1$, $**P<0.05$, $***P<0.02$).

Figure 8: The mean (±SEM) extrahepatic splanchnic (upper panel), hepatic (middle panel) and splanchnic (lower panel) balances for 5 glucogenic amino acids during the 30 min basal period and the 3 hours of high dose amino acid infusion. Asterisks (****) indicate significant differences ($P<0.01$, paired t-test) between balance observed in the basal state and during amino acid infusion.

Figure 9: Upper panels: the mean (±SEM) plasma glucose concentration in the hepatic vein, artery, and portal vein in the basal state and for 3 hours following low dose (left side) on high dose (right side) amino acid infusion. Lower panel: mean (±SEM) hepatic plasma flow during low dose (left panel) and high dose (right panel) amino acid infusion.

Figure 10: The mean (±SEM) extra hepatic splanchnic (upper panel), hepatic (middle panel), and splanchnic (lower panel) glucose balances in the basal state and during 3 hours of low dose (left) or high dose (right) amino acid infusion. Asterisk indicates values significantly ($p=0.05$ or less) different than postabsorptive value shown by the broken line.

Figure 11: The systemic rates of glucose appearance (upper panel), disappearance (middle panel), and metabolic clearance (lower panel) in 4 dogs in the basal state (broken line) and during the 3 hours of high dose amino acid infusion. Asterisk indicates values different than basal ($p<0.05$). Error bars indicate 1 SEM.

Figure 12: The mean (±SEM) hepatic balances for 4 glucogenic amino acids (upper panel), and for the individual and combined branched chain amino acids (lower panel) in the basal state and during the 3 hours of low dose infusion. Significant differences between balances in the postabsorptive and amino acid infusion periods are indicated by askerisks (*$P<0.05$, **$P<0.02$, ***$P<0.01$).

Figure 13: The mean (±SEM) of the hepatic extraction ratios of four glucogenic amino acids (upper panel), and of the individual

and combined branched chain amino acids (lower panel) obtained during the three hours following amino acid ingestion in 9 dogs (open bars), or during the last two hours of either low dose (five dogs, hatched bars) or high dose (five dogs, shaded bars) intravenous amino acids.

FIGURE 1

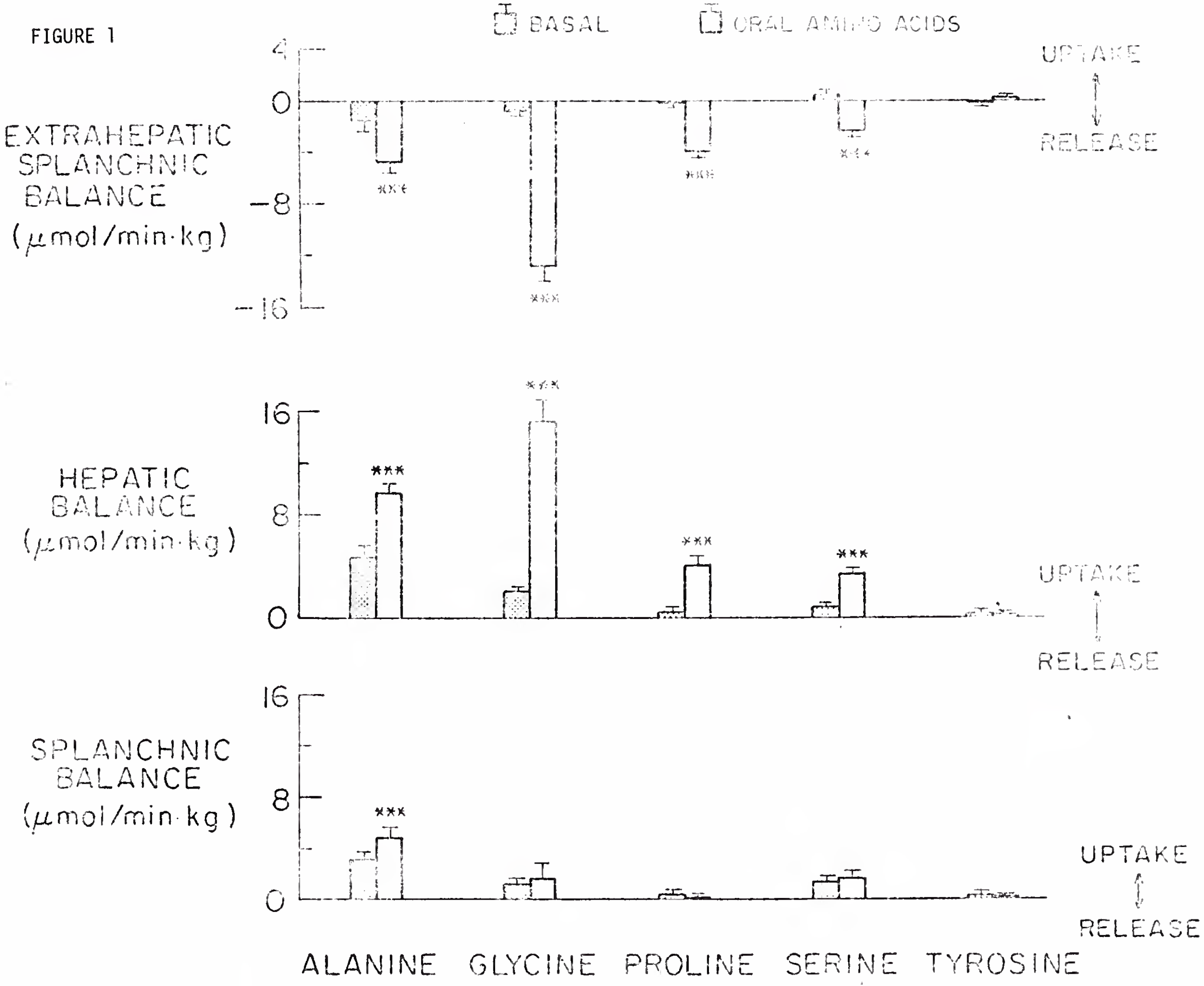
BASAL
ORAL AMINO ACIDS
EXTRAHEPATIC SPLANCHNIC BALANCE (μmol/min·kg)
4
0
−8
−16
UPTAKE
RELEASE
***
***
***
***
HEPATIC BALANCE (μmol/min·kg)
16
8
0
***
***
***
***
UPTAKE
RELEASE
SPLANCHNIC BALANCE (μmol/min·kg)
16
8
0
***
UPTAKE
RELEASE
ALANINE
GLYCINE
PROLINE
SERINE
TYROSINE

FIGURE 2

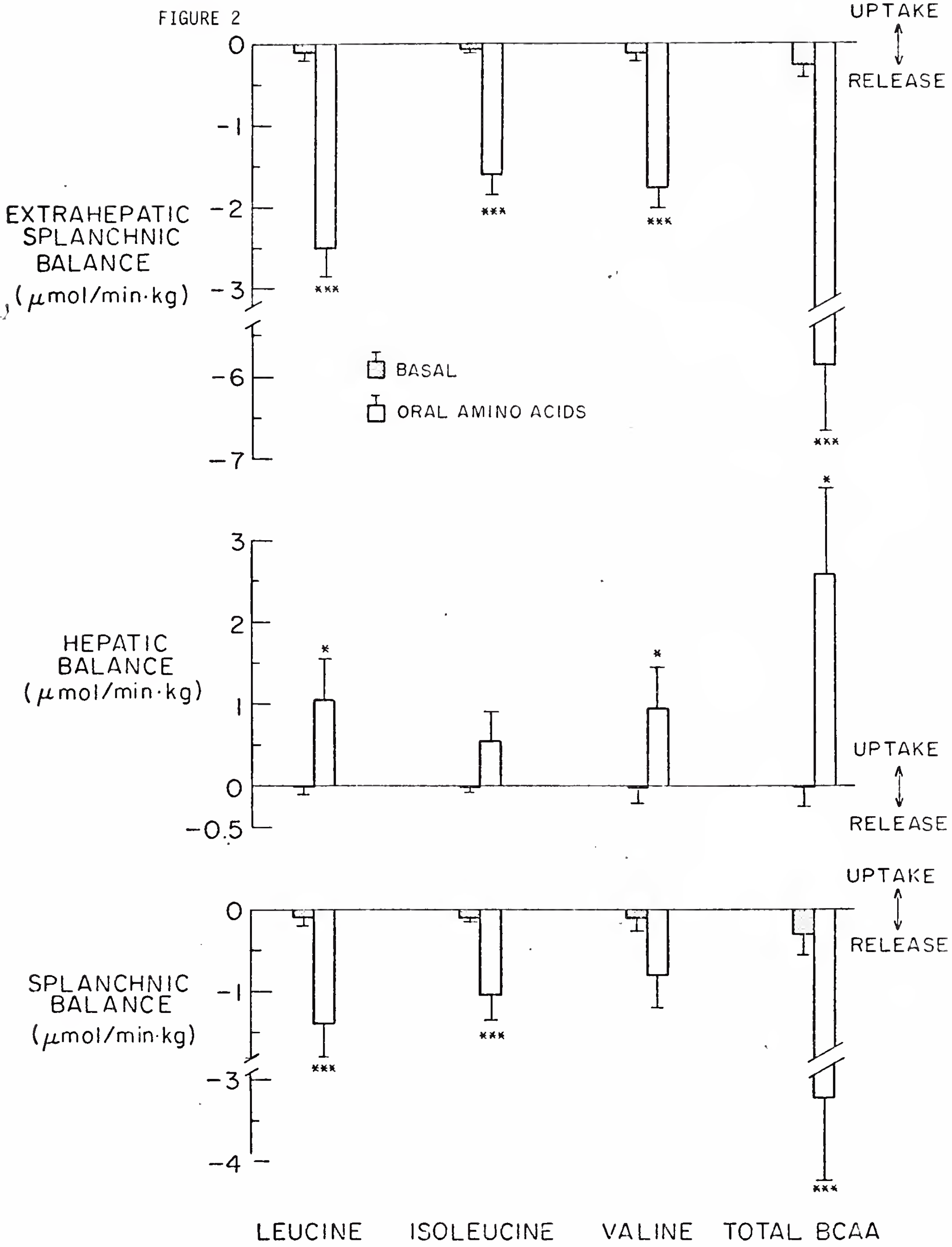
UPTAKE
RELEASE
EXTRAHEPATIC SPLANCHNIC BALANCE (μmol/min·kg)
0
-1
-2
-3
-6
-7
***
***
***
***
BASAL
ORAL AMINO ACIDS
HEPATIC BALANCE (μmol/min·kg)
3
2
1
0
-0.5
*
*
*
UPTAKE
RELEASE
UPTAKE
RELEASE
SPLANCHNIC BALANCE (μmol/min·kg)
0
-1
-3
-4
***
***
***
LEUCINE
ISOLEUCINE
VALINE
TOTAL BCAA

FIGURE 3

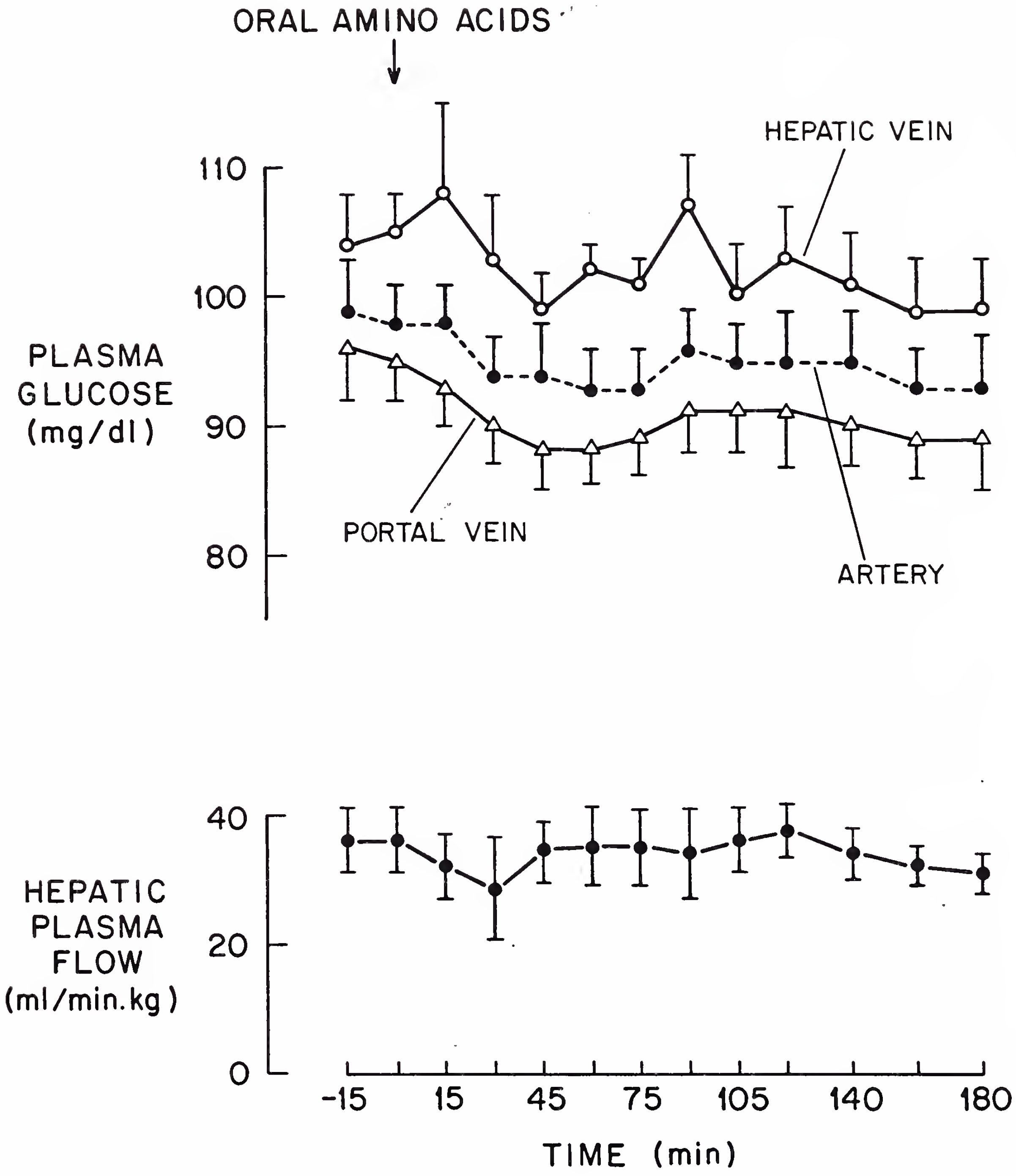
ORAL AMINO ACIDS
HEPATIC VEIN
PORTAL VEIN
ARTERY
PLASMA GLUCOSE (mg/dl)
110
100
90
80
HEPATIC PLASMA FLOW (ml/min.kg)
40
20
0
-15
15
45
75
105
140
180
TIME (min)

FIGURE 4

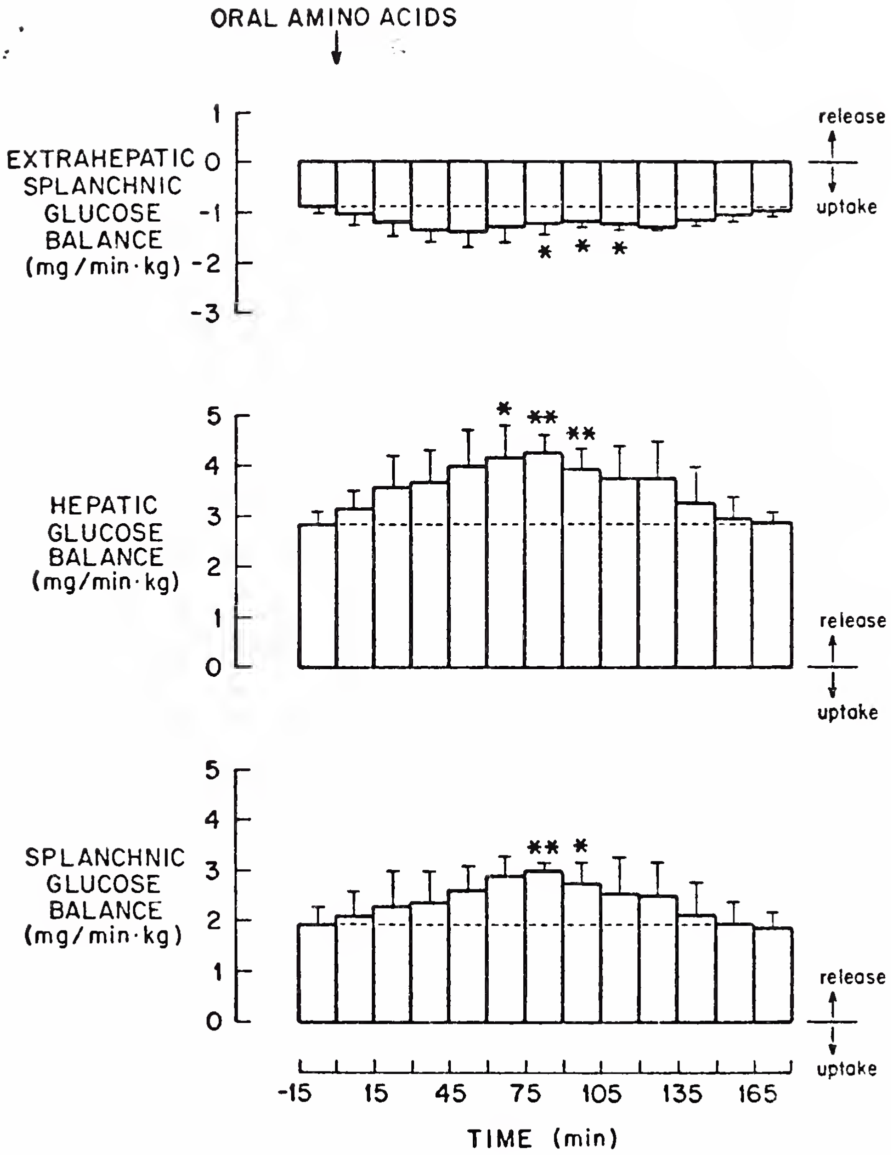
ORAL AMINO ACIDS
EXTRAHEPATIC SPLANCHNIC GLUCOSE BALANCE (mg/min·kg)
1
0
-1
-2
-3
release
uptake
* * *
HEPATIC GLUCOSE BALANCE (mg/min·kg)
5
4
3
2
1
0
* ** **
release
uptake
SPLANCHNIC GLUCOSE BALANCE (mg/min·kg)
5
4
3
2
1
0
** *
release
uptake
-15
15
45
75
105
135
165
TIME (min)

FIGURE 5

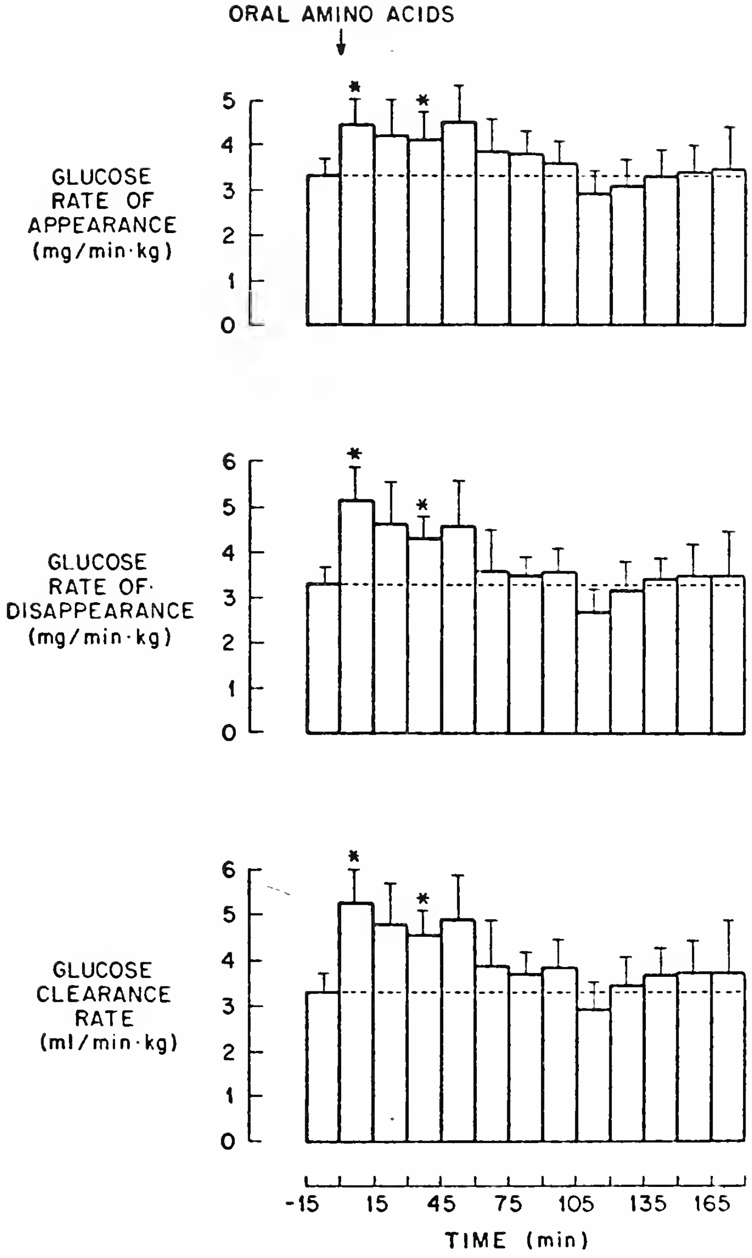
ORAL AMINO ACIDS
GLUCOSE RATE OF APPEARANCE (mg/min·kg)
0
1
2
3
4
5
GLUCOSE RATE OF DISAPPEARANCE (mg/min·kg)
0
1
2
3
4
5
6
GLUCOSE CLEARANCE RATE (ml/min·kg)
0
1
2
3
4
5
6
*
*
-15
15
45
75
105
135
165
TIME (min)

FIGURE 6

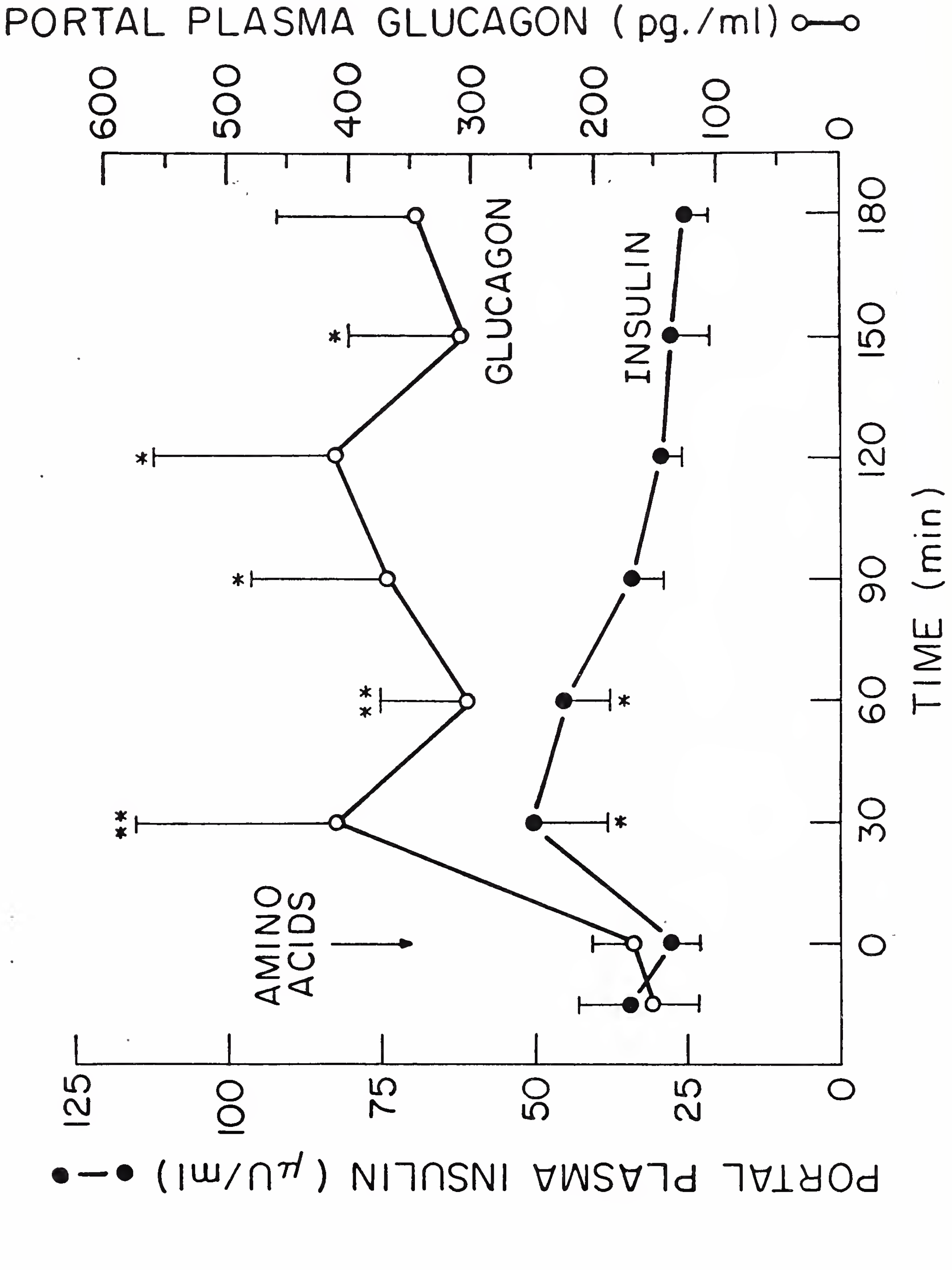
PORTAL PLASMA GLUCAGON (pg./ml) o—o
600
500
400
300
200
100
0
GLUCAGON
INSULIN
AMINO ACIDS
**
**
*
*
*
*
*
0
30
60
90
120
150
180
TIME (min)
125
100
75
50
25
0
PORTAL PLASMA INSULIN (μU/ml) ●—●

FIGURE 7

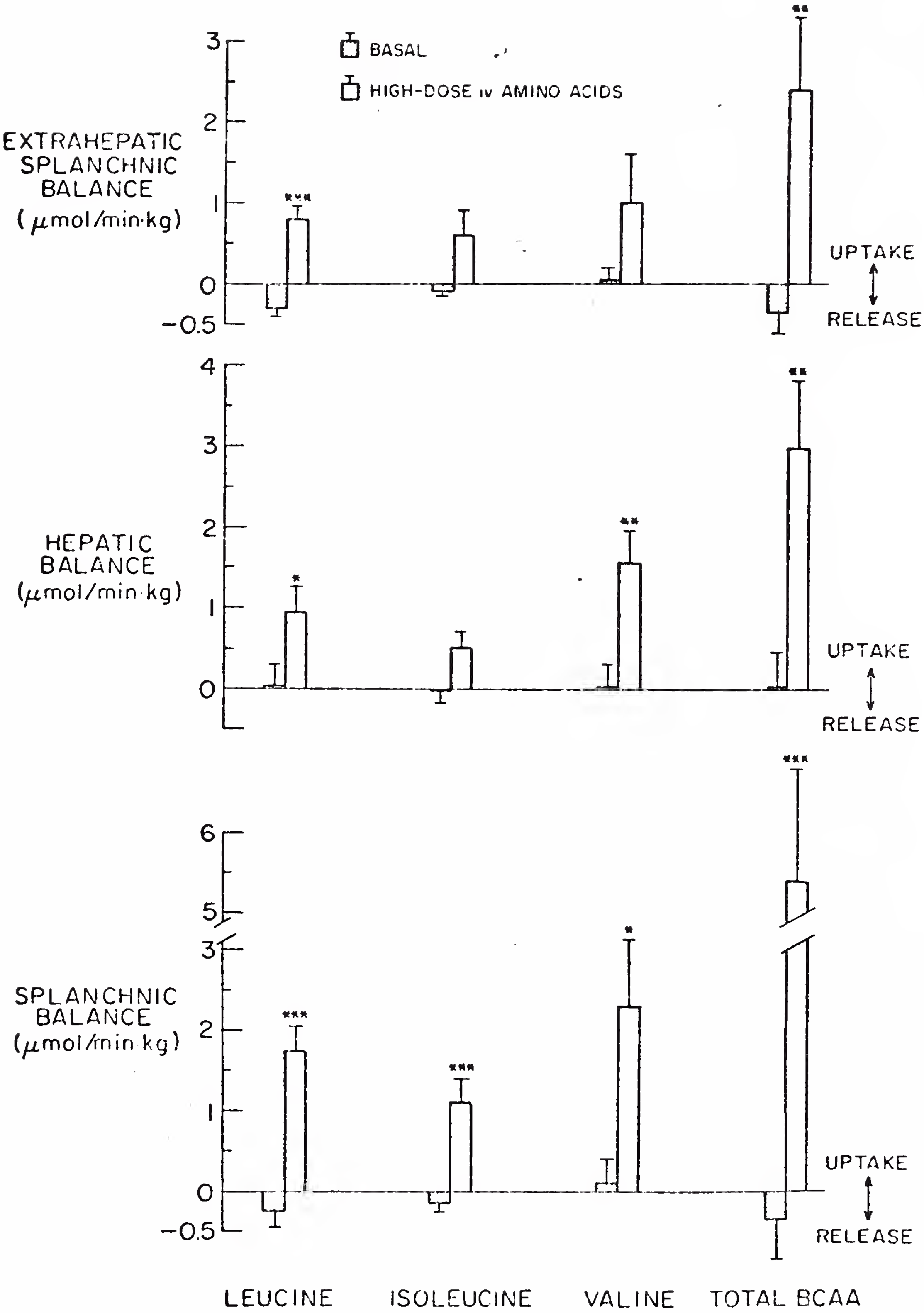
BASAL
HIGH-DOSE iv AMINO ACIDS
EXTRAHEPATIC SPLANCHNIC BALANCE (μmol/min·kg)
3
2
1
0
−0.5
***
**
UPTAKE
RELEASE
HEPATIC BALANCE (μmol/min·kg)
4
3
2
1
0
*
**
**
UPTAKE
RELEASE
SPLANCHNIC BALANCE (μmol/min·kg)
6
5
3
2
1
0
−0.5
***
***
*
***
UPTAKE
RELEASE
LEUCINE
ISOLEUCINE
VALINE
TOTAL BCAA

FIGURE 8

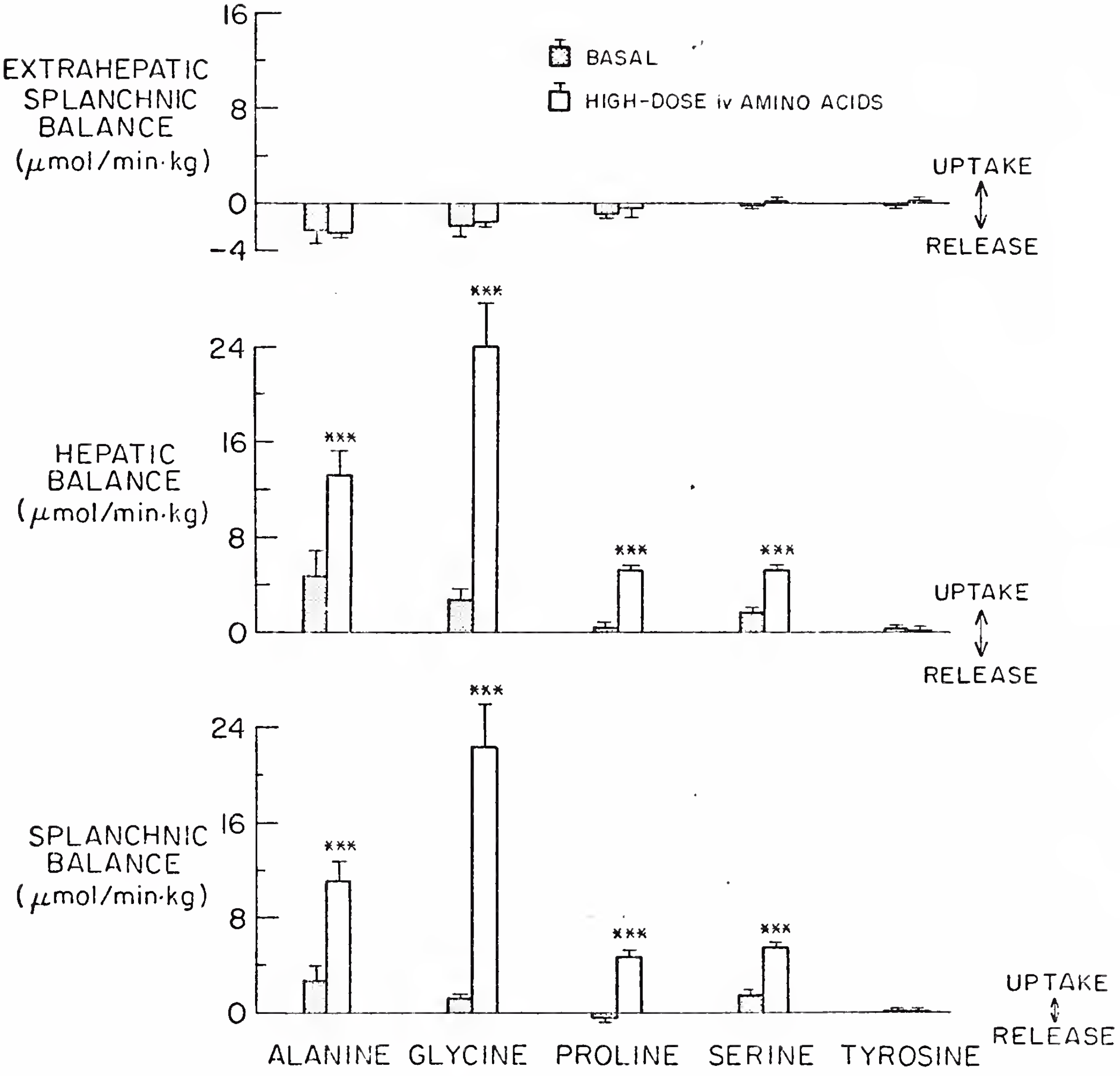
16
EXTRAHEPATIC SPLANCHNIC BALANCE (μmol/min·kg)
8
0
-4
BASAL
HIGH-DOSE iv AMINO ACIDS
UPTAKE
RELEASE
***
24
16
HEPATIC BALANCE (μmol/min·kg)
8
0
***
***
***
UPTAKE
RELEASE
***
24
16
SPLANCHNIC BALANCE (μmol/min·kg)
8
0
***
***
***
UPTAKE
RELEASE
ALANINE
GLYCINE
PROLINE
SERINE
TYROSINE

FIGURE 9

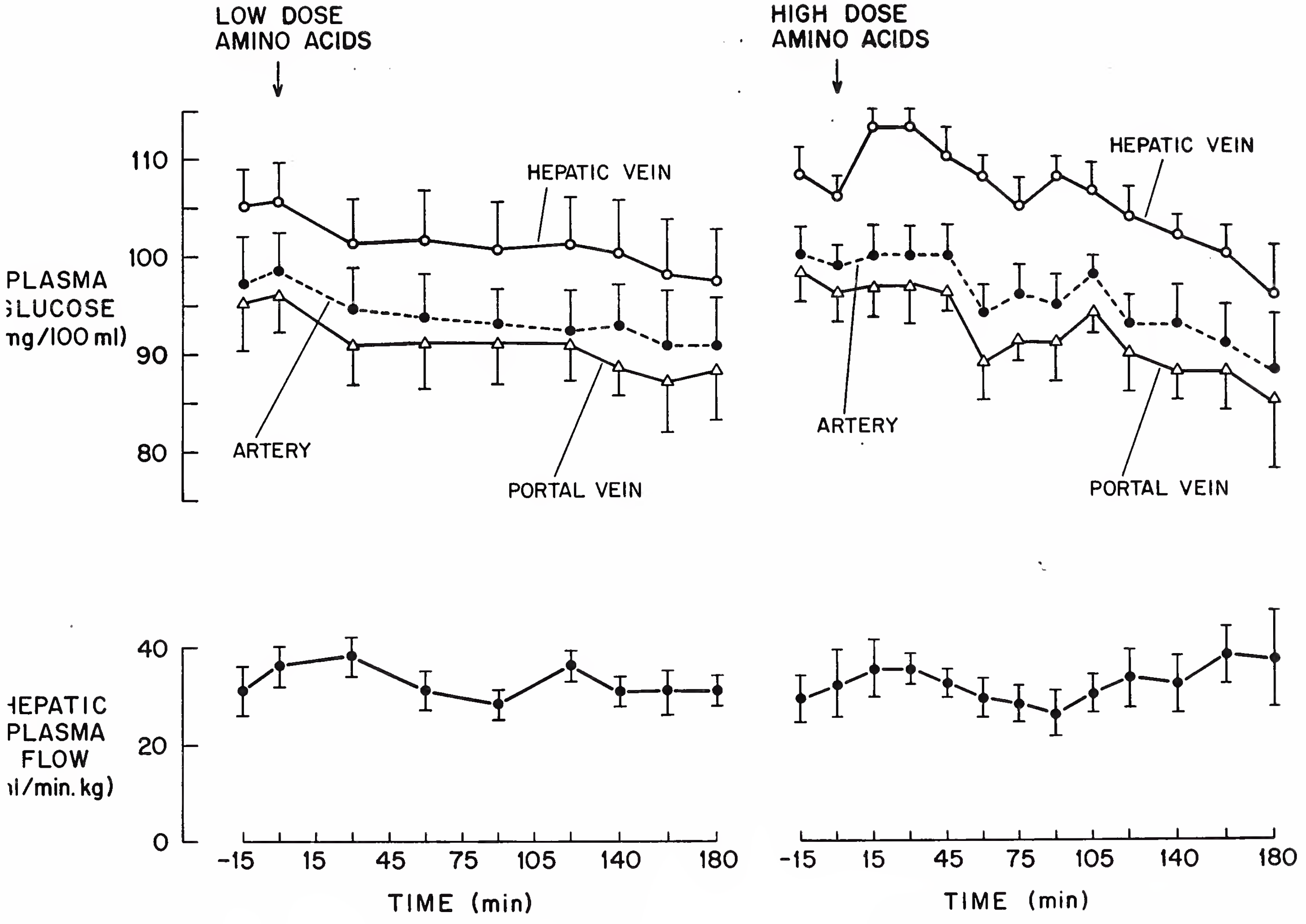
LOW DOSE
AMINO ACIDS
HIGH DOSE
AMINO ACIDS
HEPATIC VEIN
ARTERY
PORTAL VEIN
HEPATIC VEIN
ARTERY
PORTAL VEIN
PLASMA
LUCOSE
ng/100 ml)
110
100
90
80
EPATIC
PLASMA
FLOW
l/min. kg)
40
20
0
-15
15
45
75
105
140
180
TIME (min)
-15
15
45
75
105
140
180
TIME (min)

FIGURE 10

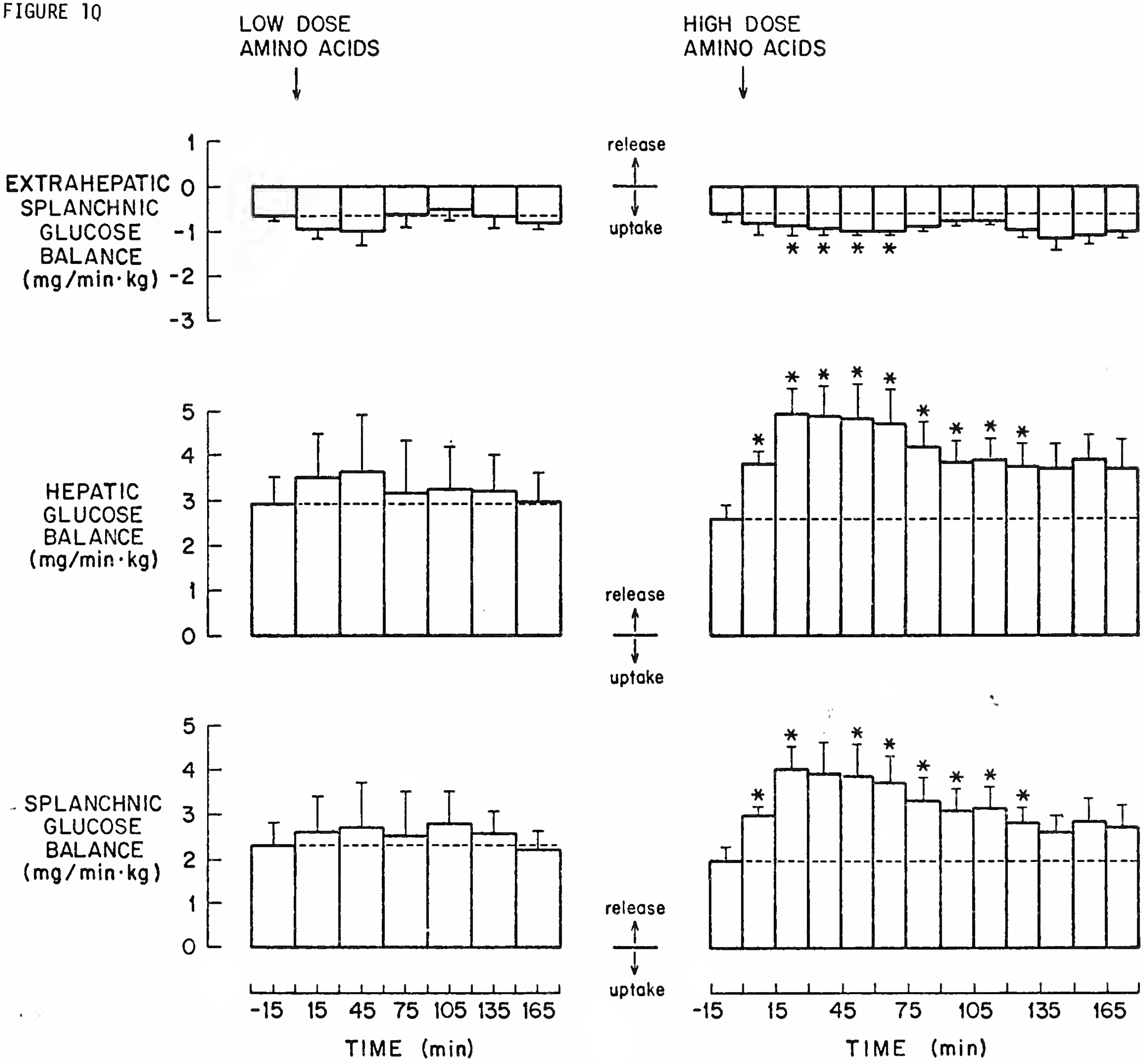
LOW DOSE AMINO ACIDS
HIGH DOSE AMINO ACIDS
EXTRAHEPATIC SPLANCHNIC GLUCOSE BALANCE (mg/min·kg)
1
0
-1
-2
-3
HEPATIC GLUCOSE BALANCE (mg/min·kg)
SPLANCHNIC GLUCOSE BALANCE (mg/min·kg)
5
4
3
2
1
0
release
uptake
*
-15
15
45
75
105
135
165
TIME (min)

FIGURE 11

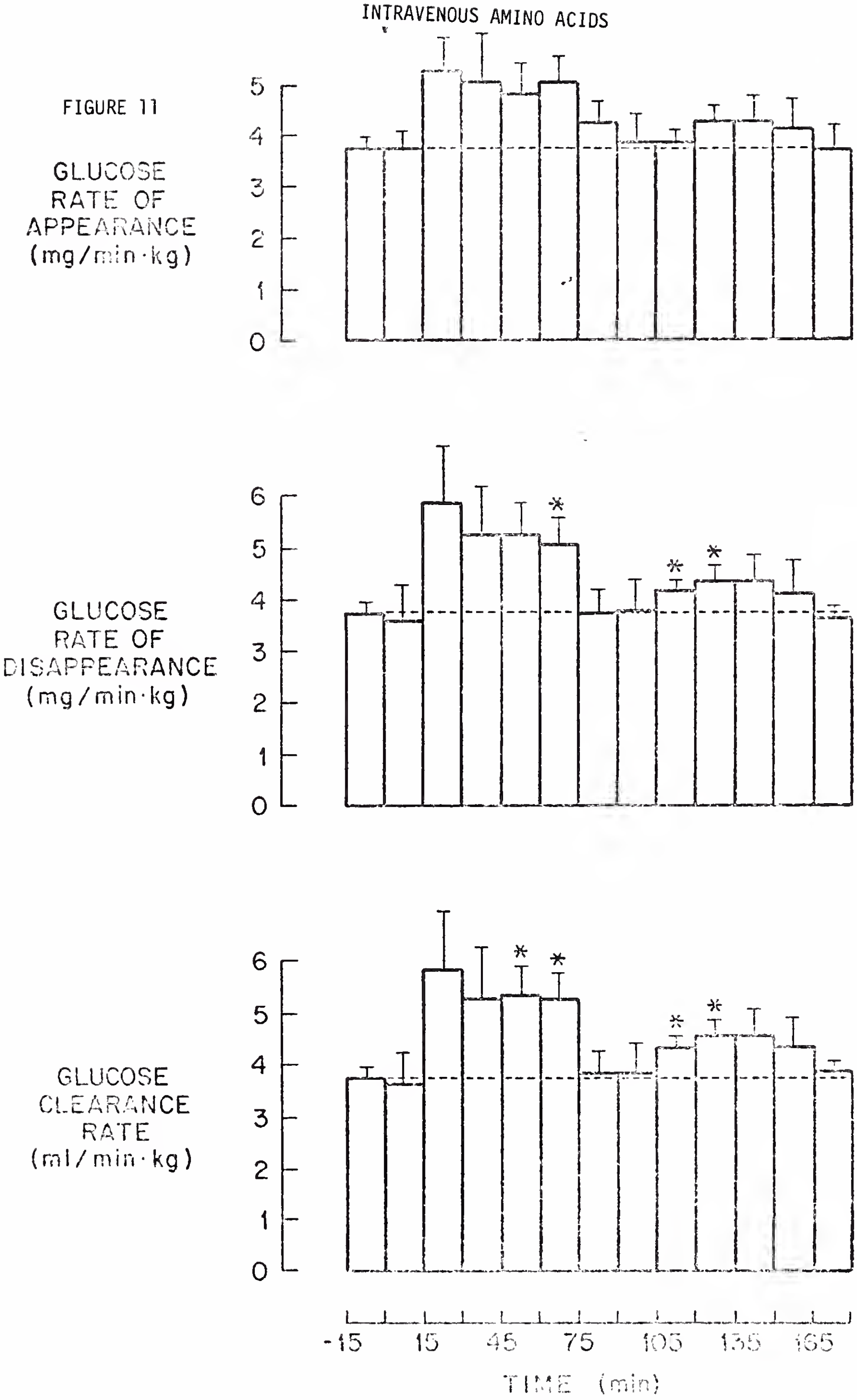
INTRAVENOUS AMINO ACIDS
GLUCOSE RATE OF APPEARANCE (mg/min·kg)
0
1
2
3
4
5
GLUCOSE RATE OF DISAPPEARANCE (mg/min·kg)
0
1
2
3
4
5
6
*
*
*
GLUCOSE CLEARANCE RATE (ml/min·kg)
0
1
2
3
4
5
6
*
*
*
*
-15
15
45
75
105
135
165
TIME (min)

FIGURE 12

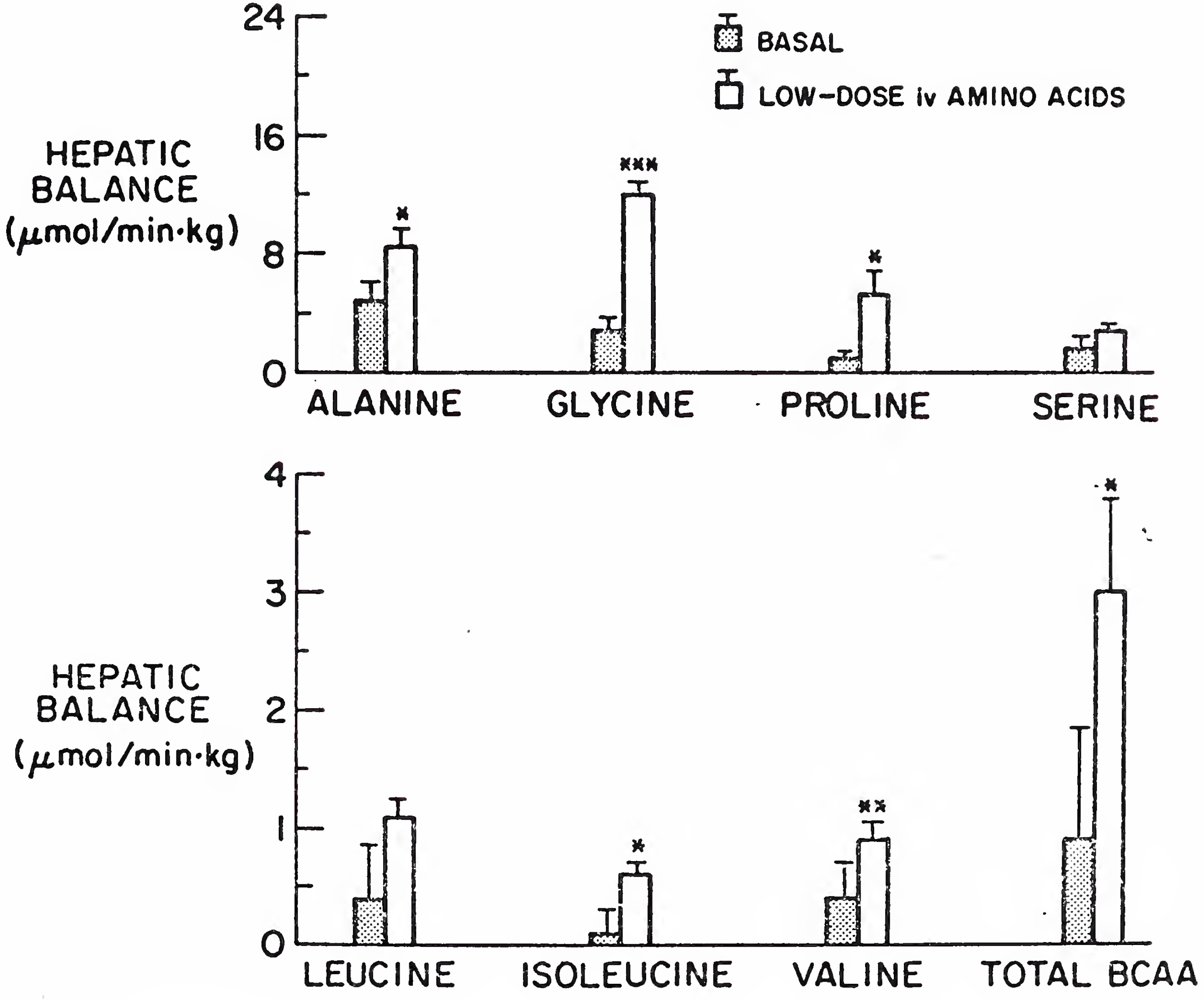
BASAL
LOW-DOSE iv AMINO ACIDS
HEPATIC BALANCE (μmol/min·kg)
24
16
8
0
ALANINE
GLYCINE
PROLINE
SERINE
*
***
*
HEPATIC BALANCE (μmol/min·kg)
4
3
2
1
0
LEUCINE
ISOLEUCINE
VALINE
TOTAL BCAA
*
**
*

FIGURE 13

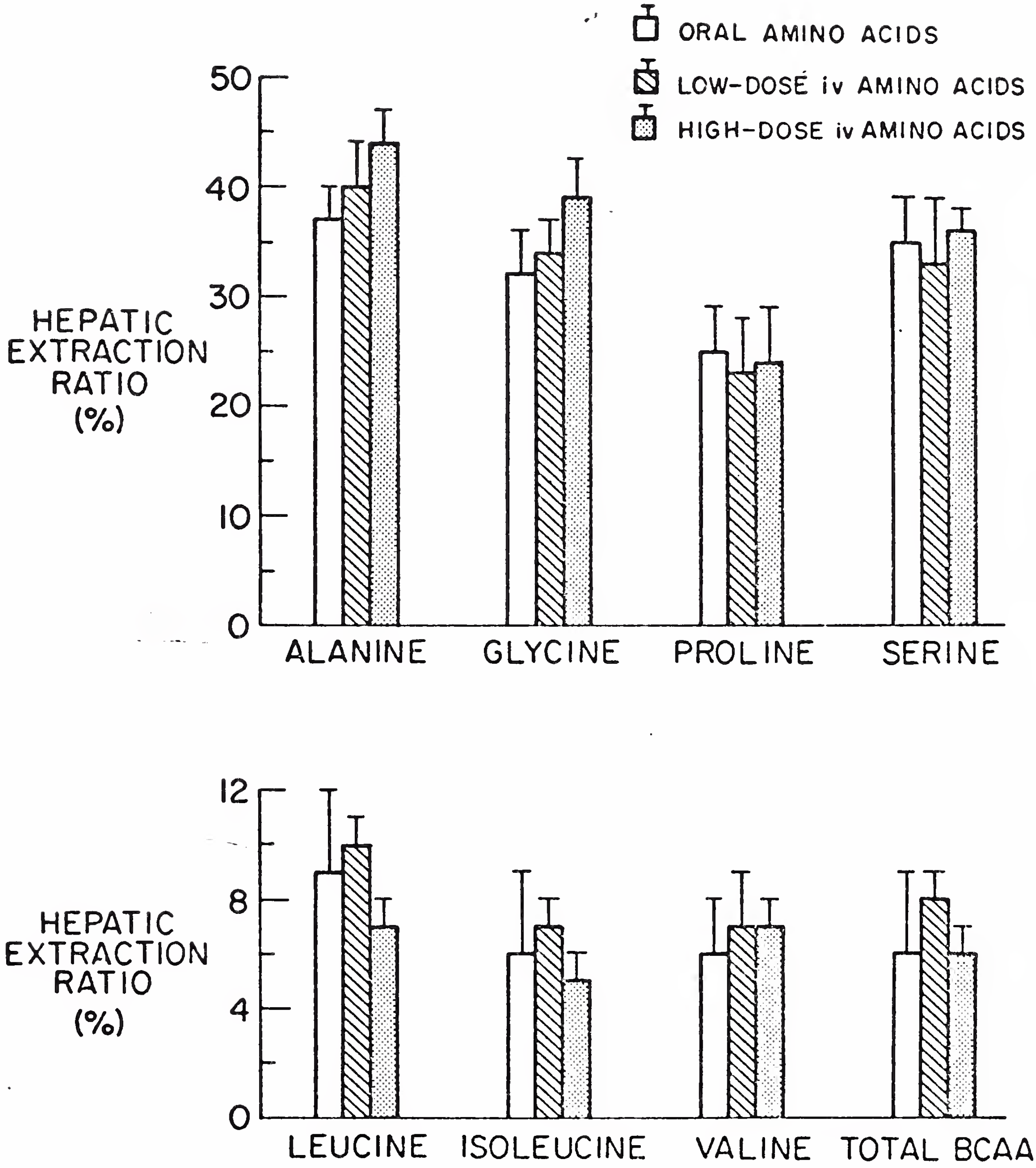
ORAL AMINO ACIDS
LOW-DOSE iv AMINO ACIDS
HIGH-DOSE iv AMINO ACIDS
HEPATIC EXTRACTION RATIO (%)
0
10
20
30
40
50
ALANINE
GLYCINE
PROLINE
SERINE
HEPATIC EXTRACTION RATIO (%)
0
4
8
12
LEUCINE
ISOLEUCINE
VALINE
TOTAL BCAA

TABLE 1 - Amino Acid concentrations in arterial, portal and hepatic venous blood in 9 dogs in the postabsorptive state and after amino acid ingestion.

		Time (min)									
		-15	0	15	30	60	90	120	140	160	180
THREONINE	a	181±41	181±40	255±63†	307±70	247±61*	282±58*	239±44†	226±46†	217±41*	202±41
	pv	181±40	169±44	324±82	367±63	304±43	315±61	270±43	250±45	229±42	219±39
	hv	168±42	159±37	266±68	296±60	255±44	271±59	227±43	220±41	213±41	196±37
SERINE	a	88±10	92±10	168±25†	219±46*	198±35§	185±34†	173±18$^{\Vert}$	159±17§	146±16§	136±15†
	pv	85±7	79±6	286±40†	323±34$^{\Vert}$	291±43$^{\Vert}$	237±31	231±18†	198±20†	174±19*	164±18$^{\Vert}$
	hv	63±7	60±8	155±30	187±33	165±28	147±25	135±15	128±13	119±14	115±16
PROLINE	a	77±7	75±8	293±45§	311±68*	296±47$^{\Vert}$	267±46	222±22$^{\Vert}$	218±33$^{\Vert}$	200±42†	166±31†
	pv	83±9	77±10	526±125	510±45§	467±68§	383±37	311±39*	278±39*	251±58	214±37§
	hv	69±8	67±8	346±56	373±47	307±55	254±33	195±28	206±31	176±27	155±31
GLYCINE	a	172±20	171±19	489±93†	712±207*	757±115$^{\Vert}$	633±138†	571±59$^{\Vert}$	560±87$^{\Vert}$	445±77§	386±74†
	pv	190±21*	184±20	1070±253*	1289±159§	1214±161$^{\Vert}$	894±140†	890±119§	771±137§	648±127§	611±120§
	hv	144±20	136±18	611±109	742±118	723±119	548±95	512±59	499±83	417±69	364±69
ALANINE	a	282±55	304±64	385±78§	460±99§	542±94$^{\Vert}$	416±53§	464±77§	459±82§	406±84*	353±82
	pv	340±62†	338±60	584±130*	653±81$^{\Vert}$	693±104$^{\Vert}$	542±80†	584±87$^{\Vert}$	552±103§	499±97$^{\Vert}$	477±99§
	hv	235±56	228±53	325±50	406±72	428±82	321±33	352±65	336±75	309±74	276±66
VALINE	a	120±4	122±4	249±35†	282±46†	290±26$^{\Vert}$	266±35§	266±26$^{\Vert}$	251±25$^{\Vert}$	237±23$^{\Vert}$	220±24§
	pv	123±4	122±6	337±68	387±30†	360±36§	306±32	304±32*	283±34	264±28*	237±24†
	hv	122±3	118±5	298±51	350±25	319±31	268±21	268±29	260±27	254±27	229±25
ISOLEUCINE	a	45±3	42±3	145±27†	164±29§	150±18$^{\Vert}$	119±14§	118±17§	113±16§	103±14§	95±16§
	pv	48±3	43±3	223±48	254±22	210±25§	162±25	160±24§	138±23†	124±20*	107±19*
	hv	48±3	43±2	167±38	225±15	172±21	135±21	133±20	128±20	121±21	104±21
LEUCINE	a	96±6	94±7	236±40†	244±47*	229±25$^{\Vert}$	173±29†	177±27§	170±26†	153±22†	142±24*
	pv	102±7	96±8	357±73	390±40†	319±34§	235±40	236±39†	208±37†	183±30*	162±27†
	hv	102±7	96±7	310±61	341±34	257±34	189±33	194±32	184±31	169±30	154±29
TYROSINE	a	36±2	32±2	50±4	48±4	45±5	40±3	33±4	36±3	35±3	30±3
	pv	38±3	34±3	50±6	48±4	40±5	43±3	36±4	36±3	37±4	34±3
	hv	31±2	29±2	50±5	49±5	39±4	39±3	33±3	34±4	33±3	30±3
PHENYLALANINE	a	36±3	37±4	63±9§	67±10§	66±7§	67±7§	59±8§	55±7§	53±6§	49±7*
	pv	40±4*	37±4	118±29	114±12*	101±12§	105±43	84±15*	72±10	71±12*	63±9$^{\Vert}$
	hv	32±3	32±4	62±9	66±7	56±6	64±6	49±7	55±11	50±7	44±8

For arterial samples p value indicates significant difference from basal (paired t).
For portal samples p value indicate significant differences from simultaneous arterial sample (paired t).

* $p < 0.05$; † $p < 0.02$; § $p < 0.01$; $^{\Vert}$ $p < 0.002$.

TABLE 2 - Splanchnic (S), extrahepatic splanchnic (EH) and hepatic (H) balances in 9 dogs in the postabsorptive state and after amino acid ingestion

		Time (min)									
		-15	0	15	30	60	90	120	140	160	180
THREONINE	S	.47±.32	.89±.27	-.67±.55	.45±2.08	-.24±.72	.26±.89	.61±.49	.54±.35	-.42±.76	.16±.39
	EH	.14±.23	.30±.50	-2.16±.99	-2.55±1.9	-2.05±.52	-1.27±.77	-1.13±.64	-.87±.36	-.45±.37	-.50±.10
	H	.32±.26	.60±.59	1.49±.74	3.00±.77	1.82±.55	1.53±.49	1.75±.52	1.41±.51	.64±.39	.66±.37
SERINE	S	1.19±.41	1.54±.43	.56±.60	1.66±.91	2.09±.78	1.91±.77	1.80±.43	1.58±.41	1.01±.35	.76±.30
	EH	.27±.33	.67±.44	-4.08±1.2†	-4.23±.8§	-3.63±.9§	-1.89±.8*	-2.34±.7||	-1.48±.6†	-.94±.37§	-1.07±.20†
	H	.91±.14	.74±.17	4.65±.9§	5.89±1.1§	5.71±1.3§	3.8±.73§	4.12±.73§	3.06±.82*	1.95±.51§	1.82±.34*
PROLINE	S	.28±.26	.47±.33	-2.27±1.0*	-2.80±2.0	-1.48±1.1	1.10±1.4	1.21±.88	.58±.39	.96±.64	.29±.55
	EH	-.32±.17	.25±.19	-6.95±2.6*	-8.34±1.8†	-4.07±2.4§	-3.59±1.1*	-3.16±1.3§	-2.27±.90§	-1.46±.69	-1.89±.44*
	H	.61±.32	.09±.33	4.68±2.0	5.55±2.2	4.88±1.2§	4.71±.63§	4.37±1.8	2.82±1.1*	2.41±1.0	2.38±.5
GLYCINE	S	1.27±.26	1.41±.31	-5.64±1.6§	-1.54±4.8	-.18±2.7	5.23±3.4	2.38±1.2	2.46±1.2	.72±.69	.36±1.2
	EH	-.70±.33	-1.02±.36	-19.8±6.2	-24.3±4.0§	-20.3±4.1||	-10.0±3.4	-13.0±3.5†	-8.59±2.5*	-6.22±1.9*	-9.11±2.4†
	H	2.05±.28	1.92±.25	13.8±6.9	21.8±4.8†	22.5±.44§	15.3±3.6†	15.4±3.4§	11.0±3.2*	6.95±1.9*	9.46±2.89*
ALANINE	S	2.19±.65	3.86±.85	3.45±2.1	2.41±1.52	5.25±1.3	4.49±.88	6.06±.96	5.55±1.2	3.81±.76	3.85±1.3
	EH	-2.46±.89	-1.05±.7	-7.53±3.2	-7.99±1.6§	-6.11±1.5§	-4.75±1.5	-4.82±.71*	-3.75±.8	-2.98±.71	-4.95±1.3*
	H	4.65±.60	4.91±.79	10.9±4.2	10.4±1.8§	11.4±1.9§	9.25±1.9§	10.9±1.6§	9.3±1.7*	6.8±.93	8.8±1.3§
VALINE	S	-.09±.12	.01±.2	-1.84±1.1	-2.84±1.2	-2.02±1.5	.25±1.2	-.17±.63	-.35±.44	-.51±.31	-.66±.33
	EH	-.11±.11	-.16±.17	-2.9±1.6	-4.4±1.1†	-2.7±.82†	-1.3±.69	-1.6±.71	-1.3±.57*	-.71±.38	-.71±.25
	H	-.01±.14	.02±.18	1.04±.64	1.57±.9	0.68±1.4	1.53±.82	1.44±.97	.96±.55	.21±.29	.01±.42
ISOLEUCINE	S	-.13±.05	-.05±.11	-1.57±.8	-3.48±1.2*	-1.42±.9	-.76±.45	-.93±.43	-.80±.24*	-.76±.27*	-.62±.34
	EH	-.08±.10	-.08±.10	-2.58±1.2	-4.52±1.2†	-2.34±.7*	-1.3±.5	-1.61±.5†	-1.0±.4*	-.71±.23*	-.53±.20*
	H	-.05±.06	.02±.09	1.07±.46	1.05±1.2	.92±.83	.54±.37	.76±.77	.20±.36	-.05±.25	-.09±.29
LEUCINE	S	-.25±.11	.09±.12	-2.91±1.2	-4.3±1.7	-1.87±1.3	-.78±.55	-1.12±.37*	-.77±.34	-.50±.32	-.81±.42
	EH	-.22±.05	0.0±.17	-4.03±1.7	-6.26±1.6†	-3.56±.95§	2.94±.78†	-2.20±.8*	-1.52±.56	-.88±.36	-.85±.26
	H	-.03±.08	.08±.13	1.11±1.2	1.95±1.5	1.69±1.1	1.16±.53	1.15±.86	.75±.54	.37±.43	.04±.43
TYROSINE	S	.26±.07	.17±.06	-.01±.12	-.02±.36	.34±.16	.01±.09	.11±.05	.16±.12	.10±.09	.04±.10
	EH	-.03±.04	-.05±.08	-.02±.13	.03±.17	.25±.12	-.13±.06	.01±.10	0.0±.04	-.07±.05	-.13±.07
	H	.30±.09	.22±.07	.01±.13	-.04±.21	.10±.12	.13±.05	.11±.08	.16±.11	.17±.09	.17±.08
PHENYLALANINE	S	.24±.06	.24±.06	.04±.08*	.12±.60	.37±.28	.26±.33	.43±.11	-.11±.34	.09±.09	.18±.10
	EH	-.13±.06	-.05±.09	-1.94±.89	-1.84±.62*	-1.36±.45†	-1.14±.38*	-.96±.41	-.70±.23*	-.51±.21	-.58±.10§
	H	.36±.06	.29±.11	1.95±.86	1.96±.43†	1.73±.33||	1.40±.16§	1.39±.43*	.60±.44	.59±.24	.76±.08†

p value refers to comparison of individual time points with the mean basal value in the same animal using paired t test. A negative sign(-)indicates net release

* $p < 0.05$; † $p < 0.02$; § $p < 0.01$; || $p < 0.002$.

TABLE 3

Hepatic extraction ratio for various amino acids in the basal state and following ingestion of an amino acid mixture.

Amino acid	Hepatic extraction ratio (%)		P value ‡
	Basal	Post-feeding	
Alanine	31 ± 5 *	37 ± 3	n.s.
Glycine	22 ± 4 *	32 ± 4	<0.025
Proline	12 ± 9	25 ± 4	<0.05
Serine	29 ± 4 *	35 ± 4	$0.1>p>0.05$
Leucine	- 1 ± 2	9 ± 3	<0.05
Isoleucine	- 3 ± 3	6 ± 3	$0.1>p>0.05$
Valine	0 ± 2	6 ± 2	<0.05
Total BCAA	- 1 ± 2	6 ± 3	<0.05

* significantly different from zero at the 1% level of significance or less.

‡ refers to comparison of mean basal vs. mean postcibal extraction ratio in 9 dogs using the paired t-test.

TABLE 4

Cumulative splanchnic, hepatic and extrahepatic splanchnic (intestinal) balance of 9 amino acids over 3 hours following an amino acid meal.

Amino acid	Oral dose (μmoles/kg)	Splanchnic balance	Hepatic balance (μmoles/kg·3 hrs)	Extrahepatic splanchnic balance
Alanine	580	891±142	1736±166	- 853±118
Serine	415	279±67	677±66	- 404±45
Glycine	1958	266±189	2523±260	-2271±185
Phenylalanine	253	33±25	189±36	- 223±33
Proline	719	-5±75	693±124	- 683±99
Tyrosine	0	19±10	16±10	- 4±9
Valine	416	-144±72	174±86	- 316±48
Leucine	512	-256±78	192±88	- 448±61
Isoleucine	390	-188±56	101±67	- 290±41
Total amino acid nitrogen	5243	+895	+6301	-5492

TABLE 5: Amino Acid concentrations in arterial, portal and hepatic venous blood in the post absorptive state and during "low-dose" amino acid infusion

		Time (min)									
		-15	0	15	30	60	90	120	140	160	180
PROLINE	a	118±18	119±15	443±113	467±110	444±95	422±122	445±80	440±95	407±85	389±120
	pv	146±25	127±21	498±104	495±100	509±86	496±81	531±93	464±13	435±122	393±94
	hv	107±14	114±19	348±81	365±103	351±73	413±94	397±86	325±57	353±73	329±97
GLYCINE	a	164±13	174±22	703±158	751±162	745±134	764±154	748±129	711±83	666±81	605±97
	pv	217±36	230±34	902±229	930±260	900±182	792±144	937±149	797±82	717±88	686±61
	hv	140±10	149±10	576±95	601±157	585±137	599±145	593±121	487±46	488±55	461±77
ALANINE	a	278±25	281±38	407±78	466±42	480±45	520±67	443±41	417±31	404±31	371±37
	pv	332±26	351±25	499±92	561±80	584±46	545±36	564±55	526±60	442±27	439±20
	hv	213±26	237±33	346±47	373±72	373±53	371±43	311±36	274±28	256±15	248±34
VALINE	a	142±19	141±16	301±5	300±14	293±26	330±42	310±25	307±17	317±16	299±27
	pv	161±17	158±20	316±24	306±30	302±27	315±36	346±31	330±18	315±36	312±20
	hv	139±15	150±24	270±18	263±26	263±26	342±64	318±36	290±12	302±11	311±26
ISOLEUCINE	a	53±6	53±6	151±25	169±18	151±25	167±30	156±20	146±9	158±10	150±19
	pv	61±6	58±8	170±39	193±34	158±26	159±27	166±13	157±11	153±22	140±15
	hv	55±4	62±10	139±22	131±18	137±16	164±36	161±27	144±9	155±10	144±21
LEUCINE	a	99±12	99±10	220±42	269±34	227±32	246±41	221±27	202±7	225±21	209±31
	pv	124±14	116±14	267±59	287±51	239±36	238±44	251±31	218±15	213±30	225±39
	hv	104±8	113±14	228±27	226±25	209±22	233±61	226±38	197±3	204±21	198±32
TYROSINE	a	46±7	45±8	54±8	60±8	51±9	56±8	50±7	50±8	50±6	48±7
	pv	58±11	50±8	53±10	57±8	51±7	52±7	58±7	55±9	52±7	51±6
	hv	41±6	44±8	54±6	52±6	46±6	52±6	48±6	44±7	46±6	39±7
PHENYLALANINE	a	58±12	56±11	96±13	114±5	106±9	113±8	102±9	100±11	102±11	97±15
	pv	60±11	63±13	131±13	125±11	115±9	115±7	127±14	113±17	111±15	107±15
	hv	51±9	50±9	81±5	87±6	81±9	90±2	81±8	81±12	80±7	83±19

Table 6: Amino Acid concentrations in arterial, portal and hepatic venous blood in the postabsorbative state and during "high dose" amino acid infusion.

		Time (min)									
		-15	0	15	30	60	90	120	140	160	180
THREONINE	a	287±90	279±91	387±66§	352±65*	352±61*	348±46*	370±63*	345±75	342±63*	336±62
	pv	270±86	279±94	377±59	361±60	341±69	361±65	349±73	357±73	361±66	336±64
	hv	246±85	257±99	328±75	305±56	304±69	283±51	309±66	284±60	309±63	285±57
SERINE	a	136±27	136±30	383±31†	353±39†	317±52	348±40*	361±49*	354±61*	350±53*	320±37*
	pv	130±30	138±33	345±29	358±28	314±52	336±43	328±44	345±54	350±48	311±39
	hv	96±22	106±27	244±29	225±16	197±28	202±15	218±22	213±29	214±34	208±26
PROLINE	a	90±19	96±21	543±32$^{\|}$	523±45§	433±48§	479±49§	466±45§	467±54§	486±70§	376±41§
	pv	112±25	113±26	528±23	557±43	455-45	476±45	434±15	476±58	489±60	384±45
	hv	105±25	92±24	432±16	414±20	334±33	342±34	346±16	351±46	359±30	292±22
GLYCINE	a	206±63	198±27	1798±216§	1629±254§	1347±239§	1361±220§	1321±230§	1313±225§	1203±255§	1011±164§
	pv	228±36	248±41	1687±214	1729±267	1414±274	1404±249	1308±231	1335±273	1248±259	1080±161
	hv	168±30	169±32	1185±157	1005±164	839±182	782±129	821±151	757±164	778±180	663±111
ALANINE	a	280±71	286±91	795±158§	751±180†	662±193*	684±177†	678±174†	692±182†	647±189*	573±153*
	pv	330±71	332±89	809±164	818±204	760±216	773±212	735±212	740±193	680±182	625±163
	hv	202±48	220±58	526±125	497±124	435±136	405±103	413±110	403±115	370±114	320±93
VALINE	a	203±37	192±31	582±79§	519±69	491±71†	517±55§	571±68$^{\|}$	540±80†	570±82$^{\|}$	546±49§
	pv	192±33	197±36	485±53	532±62	494±75	505±57	501±52	520±97	547±82	496±62
	hv	193±34	194±36	489±66	477±64	483±96	459±49	508±53	482±79	492±80	471±59
ISOLEUCINE	a	58±15	58±13	264±24$^{\|}$	256±41§	209±33†	202±19$^{\|}$	245±30§	228±38†	241±38†	203±7$^{\|}$
	pv	59±15	62±14	228±19	239±31	191±19	194±16	204±22	199±25	205±16	199±21
	hv	61±13	62±12	234±24	225±29	205±20	195±15	207±19	189±25	201±22	173±10
LEUCINE	a	116±22	114±22	435±25$^{\|}$	405±45§	310±30§	301±19$^{\|}$	333±33†	320±40†	324±43*	274±13§
	pv	120±22	124±24	385±16	392±43	294±24	296±18	291±33	294±31	290±22	255±17
	hv	122-19	122±19	386±24	364±38	293±30	269±22	279±29	276±35	280±33	233±12
TYROSINE	a	35±3	38±2	49±7	49±7	42±8	44±5	48±9	47±12	46±10	41±7
	pv	38±4	40±3	44±9	41±5	42±5	42±5	41±4	42±7	43±8	43±8
	hv	32±3	34±4	52±5	44±4	41±6	42±4	46±6	42±9	42±7	38±4
PHENYLALANINE	a	49±11	56±11	158±14$^{\|}$	157±16§	137±21*	152±12§	155±18§	142±18§	150±20§	135±9§
	pv	47±7	51±7	155±13	161±11	142±16	160±14	147±6	147±15	157±15	134±9
	hv	40±6	41±6	93±7	93±7	93±11	96±6	99±6	94±10	95±5	82±3

For arterial samples p values indicates significant difference from basal (paired t).

* $p < 0.05$; † $p < 0.02$; § $p < 0.01$; $^{\|}$ $p < 0.002$

Table 7 - Hepatic extraction ratio for amino acids in the postabsorptive state and during the last 2 hours of high-dose amino acid infusion.

Amino Acid	Hepatic Extraction Ratio %		P value (postabsorptive vs infusion)
	Postabsorptive	Infusion	
Alanine	35±6	44±3	n.s.
Glycine	27±4	39±3	n.s.
Proline	11±4	24±5	< 0.02
Serine	24±6	36±2	0.10 > P >0.05
Leucine	-3±2	7±1	< 0.05
Isoleucine	-7±3	5±1	< 0.05
Valine	1±4	7±1	n.s.
Total BCAA	-3±2	6±1	< 0.05

TABLE 8

Cumulative splanchnic, hepatic and extrahepatic splanchnic disposal of glucogenic and branched chain amino acids during high dose infusion in 5 dogs.

Amino Acid	Dose Administered	Splanchnic Balance	Hepatic Balance	Extrahepatic Splanchnic Balance
	μmoles/kg 3h			
Alanine	1160	1995±299	2364±366	-410±91*
Glycine	3916	4028±668	4329±680	-301±58
Phenylalanine	506	383±25	432±30	-59±38
Serine	830	1004±35	945±53	58±39
Proline	1438	839±76	917±110	-78±121
Tyrosine	0	10±15	3±12	4±21
Total glucogenic amino acids	7850	8256±1007	9090±970	-782±222
Leucine	1026	315±58	167±54	147±25
Isoleucine	780	197±56	91±33	106±54
Valine	832	460±156	278±65	183±105
Total BCAA	2638	973±253	536±147	436±162

A negative sign indicates a net release of amino acid

TABLE 9

Rate of continuous infusion of 14 individual amino acids during low-dose infusion

	Amino Acid	Infusion Rate μmoles/min.kg body wt.
	Alanine	2.83
	Glycine	9.55
	Serine	2.03
	Phenylalanine	1.24
	Leucine	2.50
	Isoleucine	1.92
	Valine	2.03
	Threonine	1.21
	Proline	3.51
*	Lysine	1.80
*	Methionine	1.28
*	Tryptophan	.27
*	Arginine	.76
*	Histidine	.66

* Blood levels of these amino acids not measured.

TABLE 10: Cumulative hepatic amino acid uptake during low-dose infusion in 5 dogs.

Amino Acid	Dose Administered	Hepatic uptake	% of administered load removed by liver
	µmoles/kg 3 hrs		
Alanine	580	1542±193	266±33
Glycine	1958	2086±175	109±8
Phenylalanine	253	256±30	101±12
Proline	719	925±260	128±35
Tyrosine	0	50±19	
Total glucogenic a.a. (ala+gly+phe+pro)	3510	4818±580	137±19
Leucine	513	199±24	39±5
Isoleucine	390	108±14	28±4
Valine	416	165±26	40±6
Total BCAA	1319	472±52	36±4

TABLE 11: Portal plasma insulin and glucagon concentrations in the postabsorptive state and during amino acid infusion.

	Low dose Amino Acids		High dose Amino Acids	
	Insulin μU/ml	Glucagon pg/ml	Insulin μU/ml	Glucagon pg/ml
Basal	35±5	217±78	35±3	162±40
+ 30 min	47±10	331±130	121±19**	540±110**
+ 60	46±15	363±120*	54±14	530±130*
+ 90	60±18	418±155	60±8*	490±140
+ 120	49±18	403±167	72±20	520±150*
+ 150	31±6	361±195	82±15*	590±170*
+ 180	32±12	362±192	53±16	500±190

Significant differences from basal values are indicated by asterisks * $p< 0.05$, ** $p< 0.02$.

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